MANAGEMENT OF NEOVASCULAR AGE-RELATED MACULAR DEGENERATION WITH RANIBIZUMAB:

Long-term outcomes and second eye outcomes

Dr Jamie Kok-Wai Chew MBBS

A thesis submitted in fulfilment of the requirements for the degree of Masters of Philosophy (Medicine)

Faculty of Medicine
University of Sydney
Sydney, AUSTRALIA
2017
Affiliations:

Save Sight Institute, University of Sydney, Sydney, Australia,

Sydney Institute of Vision Science, Sydney, Australia

Statement of originality:
This is to certify that to the best of my knowledge, the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes.

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.
ACKNOWLEDGEMENTS

My sincerest thanks and gratitude to my primary supervisor Andrew Chang and co-supervisor Meidong Zhu for enlightening me to possibilities that I had not considered before, encouraging a culture of learning from each other and together as a research group, and their invaluable and generous guidance, dedication and support throughout my entire candidature.

Thanks to my fellow researchers Haitao, Geoff, Thomas, Adil and Nichole for their expert advise and help, to all of the staff at Sydney Retina Clinic for their assistance, and to Kehui Luo for her assistance and statistical expertise.

Finally I wish to thank my parents Soong-Yeow and Jee-Chin, sister Emily and Anastasia for all of their love and support.

Images presented in this thesis:

Unless otherwise specified, all clinical images were obtained at Sydney Retina Clinic, Sydney, New South Wales, AUSTRALIA.
# TABLE OF CONTENTS

TABLE OF CONTENTS ......................................................................................... 2  
LIST OF FIGURES .................................................................................................. 9  
LIST OF TABLES ..................................................................................................... 11  
LIST OF ABBREVIATIONS ................................................................................... 12  
PUBLICATIONS ARISING FROM THIS THESIS ............................................... 14  
PUBLISHED ABSTRACTS FROM THIS THESIS ............................................... 15  
ABSTRACT ........................................................................................................... 16  

**CHAPTER ONE - Literature review** ................................................................. 20  

1.1 - Introduction ................................................................................................ 20  

1.2 - Definition of age-related macular degeneration ........................................ 21  

1.3 - Classification of age-related macular degeneration ..................................... 23  
   1.3.1 - Dry age-related macular degeneration .................................................. 23  
   1.3.2 – Neovascular age-related macular degeneration ..................................... 25  
1.3.2 - Grading the severity of age-related macular degeneration ....................... 28  
   1.3.3 - Classification of neovascular age-related macular degeneration .......... 30  

1.4 Epidemiology .................................................................................................. 40  
   1.4.1 - The global impact of macular degeneration .......................................... 40  
   1.4.2 - Macular degeneration in Australia: .................................................... 41  
   1.4.3 - Incidence of second eye neovascular age-related macular degeneration .......................................................... 45  
   1.4.4 - Timing of neovascular age-related macular degeneration developing in  
   the second eye relative to the first eye .......................................................... 46  

1.5 - Population screening for age-related macular degeneration ....................... 47  
1.6 - Ocular risk factors for neovascular age-related macular degeneration: 49
1.6.1 - Ocular signs of age-related macular degeneration observed on clinical examination: ................................................................. 49

1.7 Natural history of neovascular age-related macular degeneration: ........ 51
  1.7.1 - Rate of visual loss: ........................................................................................................ 52
  1.7.2 - Risk of blindness after reporting symptoms ......................................................... 53

1.8 - Clinical presentation of age-related macular degeneration ................. 55
  1.8.1 - Symptoms of neovascular age-related macular degeneration: ........ 55
  1.8.2 - Signs of neovascular age-related macular degeneration ..................... 58

1.9 - Diagnosis of neovascular age-related macular degeneration ............ 61
  1.9.1 - Fundus fluorescein angiographic findings in neovascular age-related macular degeneration ........................................................................ 64
  1.9.2 - Optical coherence tomography in neovascular age-related macular degeneration ..................................................................................... 65
  1.9.3 – Indocyanine green angiography ................................................................. 72

1.10 – History of treatment of neovascular age-related macular degeneration ..................................................................................................................... 75
  1.10.1 - Thermal laser ................................................................................................. 75
  1.10.2 - Photodynamic therapy .................................................................................. 76
  1.10.3 - Other treatments less widely adopted ...................................................... 77

1.11 - History of vascular endothelial growth factor and retinal disease ...... 79
  1.11.1 Role of vascular endothelial growth factor - physiological .................. 80
  1.11.2 - Role of vascular endothelial growth factor - pathological .................. 80

1.12 Anti-vascular endothelial growth factor agents ......................................... 82
  1.12.1 - Pegaptanib .................................................................................................. 83
  1.12.2 - Bevacizumab ............................................................................................. 84
  1.12.3 - Ranibizumab ............................................................................................. 85
  1.12.3.1 – The evolution of ranibizumab treatment regimes ......................... 86
  1.12.3.1.4 - Treat and extend regime ............................................................... 91
1.12.4 - Aflibercept ........................................................................................................... 92
1.12.5 – Continuing challenges of anti-VEGF therapy ........................................... 94

1.13 - Safety concerns of anti-vascular endothelial growth factor agents and
its administration........................................................................................................... 95
  1.13.1 – Anti-vascular endothelial growth factor crossing into the systemic
circulation, and the differences between ranibizumab and bevacizumab: .......... 95
  1.13.2 - Systemic adverse events related to ranibizumab ........................................ 96
  1.13.3 - Ocular adverse events ................................................................................. 97
  1.13.4 - Measures to decrease the incidence of endophthalmitis ......................... 98

1.14 - Long – term outcomes of ranibizumab therapy............................................. 99

1.15 - Other issues surrounding management of neovascular age-related
macular degeneration with anti-vascular endothelial growth factor agents 100
  1.15.1 - Does anti-vascular endothelial growth factor treatment in the first eye
result in a benefit to the second eye? ....................................................................... 100
  1.15.2 - The rate of recurrence of inactive disease .................................................. 102

1.16 - Anti vascular endothelial growth factor agents - economic
considerations .............................................................................................................. 103
  1.16.1 - Direct costs to the health system ................................................................. 103
  1.16.2 - Neovascular age-related macular degeneration related legal blindness
.................................................................................................................................. 104
  1.16.3 - Worldwide access to ranibizumab ............................................................... 105

1.17 - Unresolved questions in neovascular age-related macular
degeneration management and thesis hypothesis ................................................. 106

1.18 - Thesis aims ....................................................................................................... 108

CHAPTER TWO – General methods for studies ....................................................... 109

2.1 - Introduction ....................................................................................................... 109

2.2 - Patient recruitment .......................................................................................... 110
  2.2.1 - Study design and establishment of the database: .................................... 110
2.2.2 - Eligibility for subsidised ranibizumab .......................................................... 110
2.2.3 – Inclusion and exclusion criteria .................................................................... 111
2.2.4 - Extension of the ranibizumab retrospective database for the second eye
ranibizumab study (SERS) .................................................................................. 112

2.3 - Clinical protocols for the treatment of neovascular age-related macular
degeneration ............................................................................................................ 114
2.3.1 - Baseline clinical assessment: ........................................................................ 114
2.3.2 - Measurement of visual acuity at baseline and all subsequent visits..... 114
2.3.3 – Optical coherence tomography image acquisition for baseline and
subsequent follow-up visits ................................................................................... 117
2.3.4 - Clinical assessments at follow-up visits ....................................................... 121
2.3.5 - Ranibizumab injection protocol and follow-up visits ............................... 122

2.4 - Source data collection ....................................................................................... 123
2.4.1 - Baseline visit for long term ranibizumab study and second eye
ranibizumab study ................................................................................................ 123
2.4.2 - Follow-up data points (including optical coherence tomography ........... 124
2.4.3 - Adverse events .............................................................................................. 124

2.5 – Optical coherence tomography data grading and analysis ..................... 125
2.6 - Fundus fluorescein angiography grading......................................................... 133
2.7 - Source data entry and verification ................................................................. 135
2.8 - Statistical methods ......................................................................................... 135

CHAPTER THREE - Long-term outcomes of intravitreal ranibizumab for
neovascular age-related macular degeneration in clinical practice.....137
3.1 - Background ..................................................................................................... 137
3.2 - Methods ........................................................................................................... 138
3.2.1 - Study design ................................................................................................. 138
3.2.2 - Patient eligibility .......................................................................................... 138
3.2.3 - Patient assessment at baseline visit and follow-up visits ..................... 138
3.2.5 - Treatment regime ................................................................. 138
3.2.6 - Optical coherence tomography analysis ................................. 139
3.2.7 - Fundus fluorescein angiography grading ................................. 139
3.2.8 - Statistical methods ................................................................ 139

3.3 - Results ...................................................................................... 140
3.3.1 - Overview: ............................................................................ 140
3.3.2 - Visual acuity .......................................................................... 144
3.3.3 - Previous treatment naïve patients ......................................... 150
3.3.4 - Central macular thickness ...................................................... 150
3.3.5 - Impact of baseline retinal fluid type on visual acuity and central macular thickness changes ................................................. 150
3.3.6 - Baseline choroidal neovascularisation type and treatment outcomes ................................................................. 151
3.3.7 - Number of injections .............................................................. 151
3.3.8 - Adverse events .................................................................... 153

3.4 - DISCUSSION .......................................................................... 156
3.4.1 - Differences in patient characteristics between real-world cohorts and clinical trials ................................................................. 156
3.4.2 - Long-term mean visual acuity ............................................... 156
3.4.3 - The relationship between baseline visual acuity and 5-year visual acuity ........................................................................... 157
3.4.4 - The relationship between the number of injections and 5-year visual acuity .............................................................. 158
3.4.5 - Anatomical outcomes ............................................................ 158
3.4.6 - Adverse events .................................................................... 161
3.4.7 - Strengths and limitations of the study ................................. 162

3.5 - Conclusions ............................................................................ 163

CHAPTER FOUR - Bilateral and sequential neovascular age-related macular degeneration: clinical presentation and treatment .............. 164
4.1 - Background .................................................................................................................. 164

4.2 - Methods ..................................................................................................................... 166
  4.2.1 - Study design .......................................................................................................... 166
  4.2.2 - Second eye assessment ......................................................................................... 166
  4.2.3 - Treatment protocol, optical coherence tomography analysis and fundus fluorescein angiography grading and statistical methods ........................................ 166

4.3 - Results ....................................................................................................................... 167
  4.3.1 - Clinical profile of second treated eyes at diagnosis ............................................. 169
  4.3.2 – Optical coherence tomography characteristics .................................................... 172
  4.3.3 - Visual acuity at baseline and at 12-months post ranibizumab therapy ............ 174

4.4 - Discussion ................................................................................................................ 177
  4.4.1 - Symptoms and visual acuity change of the second eye at baseline ..... 177
  4.4.2 – Visual acuity change of second eyes over 12-months ........................................ 179
  4.4.3 - Anatomical outcomes of second eyes compared to first eyes ....................... 179
  4.4.4 - Study strengths and limitations ........................................................................ 181

4.5 - Conclusions .............................................................................................................. 182

CHAPTER FIVE - General discussions and conclusions .............................................. 183

  5.1 - Key findings and implications of the thesis research ........................................... 183
    5.1.1 - Long-term ranibizumab study .......................................................................... 183
    5.1.2 - Second eye ranibizumab study ....................................................................... 185

  5.2 - Challenges and limitations .................................................................................... 187
    5.2.1 – Optical coherence tomography evolution of technology ................................. 187
    5.2.2 - Retrospective design and missing data ............................................................. 188

  5.3 - Future directions for research ................................................................................ 189

  5.4 - Conclusions of this thesis ....................................................................................... 190

APPENDICES .................................................................................................................... 191

  Long-term ranibizumab study baseline data collection sheet ............................................... 192
Long-term ranibizumab study - follow-up data collection sheet .................. 195
Long-term ranibizumab study – adverse events data collection sheet ...... 199
Long-term ranibizumab study – OCT grading data collection sheet .......... 200
Second eye ranibizumab study baseline data collection sheet ................. 201
Second eye ranibizumab study follow-up data collection sheet ............... 202

REFERENCES .................................................................................................................. 204
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Schematic diagram of macular degeneration</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>Dry age-related macular degeneration images</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>Geographic atrophy images</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>Neovascular age-related macular degeneration fundus images</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>Classification of choroidal neovascularisation based on angiographic appearance</td>
<td>31</td>
</tr>
<tr>
<td>6</td>
<td>Occult choroidal neovascularisation imaging</td>
<td>33</td>
</tr>
<tr>
<td>7</td>
<td>Classic choroidal neovascularisation imaging</td>
<td>35</td>
</tr>
<tr>
<td>8</td>
<td>Retinal angiomaticous proliferation: choroidal neovascularisation imaging</td>
<td>37</td>
</tr>
<tr>
<td>9</td>
<td>Prevalence of age-related macular degeneration in Australia</td>
<td>42</td>
</tr>
<tr>
<td>10</td>
<td>Amsler grid</td>
<td>47</td>
</tr>
<tr>
<td>11</td>
<td>Risk of progression to late age-related macular degeneration</td>
<td>49</td>
</tr>
<tr>
<td>12</td>
<td>Duration of symptomatic neovascular age-related macular degeneration and percentage of cases with legal blindness</td>
<td>53</td>
</tr>
<tr>
<td>13</td>
<td>Neovascular age-related macular degeneration patient with a central scotoma</td>
<td>56</td>
</tr>
<tr>
<td>14</td>
<td>Macular haemorrhage secondary to neovascular age-related macular degeneration</td>
<td>58</td>
</tr>
<tr>
<td>15</td>
<td>Regressing calcified drusen and pigmentary changes at the macula</td>
<td>59</td>
</tr>
<tr>
<td>16</td>
<td>Optical coherence tomography-angiography and neovascular age-related macular degeneration</td>
<td>62</td>
</tr>
<tr>
<td>17</td>
<td>Fibrovascular pigment epithelial detachment</td>
<td>68</td>
</tr>
<tr>
<td>18</td>
<td>Serous pigment epithelial detachment</td>
<td>69</td>
</tr>
<tr>
<td>19</td>
<td>Retinal angiomaticous proliferation</td>
<td>70</td>
</tr>
<tr>
<td>20</td>
<td>Polypoidal choroidal vasculopathy diagnosis with indocyanine green angiography</td>
<td>72</td>
</tr>
<tr>
<td>21</td>
<td>Treatment response seen with indocyanine green angiography</td>
<td>73</td>
</tr>
<tr>
<td>22</td>
<td>Cirrus optical coherence tomography scanner</td>
<td>117</td>
</tr>
</tbody>
</table>
Figure 23 - Spectralis Heidelberg retinal angiograph

Figure 24 - Fluorescein dye

Figure 25 - Stratus optical coherence tomography showing poor automated segmentation borders

Figure 26 - Stratus optical coherence tomography with corrected segmentation borders

Figure 27 - Stratus optical coherence tomography with a decentred foveal pit 1/3

Figure 28 - Stratus optical coherence tomography with a decentred foveal pit 2/3

Figure 29 - Stratus optical coherence tomography with a decentred foveal pit 3/3

Figure 30 - Fundus fluorescein angiography showing neovascular age-related macular degeneration involvement of the foveal avascular zone

Figure 31 - Study population for the long-term ranibizumab study

Figure 32 - Change in visual acuity associated with ranibizumab treatment over a 5-year period

Figure 33 - Proportions of patients with visual acuity change

Figure 34 - Number of injections required per year

Figure 35 - Clinical presentation of second eye neovascular age-related macular degeneration

Figure 36 - Case Study: asymptomatic second eye neovascular age-related macular degeneration with good visual acuity

Figure 37 - Mean visual acuity change of first vs. second eyes in the second eye ranibizumab study
LIST OF TABLES

Table 1 - Age-related macular degeneration severity and key features 28
Table 2 - Summary of age-related macular degeneration treatment in Australia 44
Table 3 - Conversion table of Snellen visual acuity to approximate early treatment of diabetic retinopathy study letters 116
Table 4 - General baseline characteristics for the long-term Ranibizumab cohort 142
Table 5 - Ocular baseline characteristics for the long-term ranibizumab study cohort 143
Table 6 - Change in visual acuity over 5-years in the long-term Ranibizumab study, sub-grouped to baseline visual acuity 147
Table 7 - Linear regression analysis of visual acuity change over 5-years in the long-term ranibizumab study 149
Table 8 - Ocular adverse events In the long-term Ranibizumab cohort 154
Table 9 - Non ocular adverse events in the long-term ranibizumab cohort 155
Table 10 - Baseline and injection characteristics of the second eye ranibizumab study cohort 168
Table 11 - Optical coherence tomography characteristics at baseline and 12-months in the second-eye ranibizumab cohort 173
Table 12 - Visual acuity stability and optical coherence tomography findings sub-grouped by good baseline visual acuity in second-eye ranibizumab study cohort 176
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE</td>
<td>Adverse events</td>
</tr>
<tr>
<td>AREDS</td>
<td>Age-related eye disease study</td>
</tr>
<tr>
<td>AMD</td>
<td>Age-related macular degeneration</td>
</tr>
<tr>
<td>ANCHOR</td>
<td>Anti-VEGF antibody for the treatment of predominantly classic choroidal neovascularization in AMD</td>
</tr>
<tr>
<td>ANOVA</td>
<td>One-way analysis of variance</td>
</tr>
<tr>
<td>AUD</td>
<td>Australian dollars</td>
</tr>
<tr>
<td>CMT</td>
<td>Central macular thickness</td>
</tr>
<tr>
<td>CNV</td>
<td>Choroidal neovascularisation</td>
</tr>
<tr>
<td>CNVM</td>
<td>Choroidal neovascular membrane</td>
</tr>
<tr>
<td>CATT</td>
<td>Comparison of age-related macular degeneration treatments trials</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>DMO</td>
<td>Diabetic macular oedema</td>
</tr>
<tr>
<td>ETDRS</td>
<td>Early treatment diabetic retinopathy study</td>
</tr>
<tr>
<td>EXCITE</td>
<td>Efficacy and safety of Ranibizumab in patients with Subfoveal choroidal neovascularization (CNV) secondary to age-related macular degeneration</td>
</tr>
<tr>
<td>EURETINA</td>
<td>European society of retina specialists</td>
</tr>
<tr>
<td>Fc</td>
<td>Fragment crystallisable</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and drug administration</td>
</tr>
<tr>
<td>FFA</td>
<td>Fundus fluorescein angiography</td>
</tr>
<tr>
<td>HARBOR</td>
<td>Phase III, double-masked, multicenter, randomized, Active treatment-controlled study of the efficacy and safety of 0.5 mg and 2.0 mg Ranibizumab administered monthly or on an as-needed Basis (PRN) in patients with subfoveal neovascular age-related macular degeneration</td>
</tr>
<tr>
<td>ICG</td>
<td>Indocyanine green</td>
</tr>
<tr>
<td>ILM</td>
<td>Inner limiting membrane</td>
</tr>
<tr>
<td>IS</td>
<td>Inner segment of photoreceptors</td>
</tr>
<tr>
<td>IOP</td>
<td>Intra-ocular pressure</td>
</tr>
<tr>
<td>IRF</td>
<td>Intra-retinal fluid</td>
</tr>
<tr>
<td>IVTA</td>
<td>Intra-vitreal triamcinolone acetate</td>
</tr>
<tr>
<td>LTRS</td>
<td>Long-term ranibizumab study</td>
</tr>
<tr>
<td>MPS</td>
<td>Macular photocoagulation study</td>
</tr>
<tr>
<td>MARINA</td>
<td>Minimally classic/Occult trial of the anti-VEGF antibody ranibizumab in the treatment of neovascular AMD</td>
</tr>
<tr>
<td>nAMD</td>
<td>Neovascular age-related macular degeneration</td>
</tr>
<tr>
<td>OCT</td>
<td>Optical coherence tomography</td>
</tr>
<tr>
<td>OCT-A</td>
<td>Optical coherence tomography angiography</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>OS</td>
<td>Outer segment of photoreceptors</td>
</tr>
<tr>
<td>PBS</td>
<td>Pharmaceutical benefits scheme</td>
</tr>
<tr>
<td>PIER</td>
<td>Phase Illb, Multicenter, Randomised, Double- Masked, Sham Injection- Controlled Study of the Efficacy and Safety of Ranibizumab</td>
</tr>
<tr>
<td>PDT</td>
<td>Photodynamic therapy</td>
</tr>
<tr>
<td>PED</td>
<td>Pigment epithelial detachment</td>
</tr>
<tr>
<td>PGF</td>
<td>Placental growth factor</td>
</tr>
<tr>
<td>PRN</td>
<td>Pro-re nata</td>
</tr>
<tr>
<td>PDR</td>
<td>Proliferative diabetic retinopathy</td>
</tr>
<tr>
<td>PRONTO</td>
<td>Prospective OCT imaging of patients with Neovascular AMD treated with Intra-ocular Lucentis</td>
</tr>
<tr>
<td>QALY</td>
<td>Quality adjusted life year</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>RAP</td>
<td>Retinal angiomatous proliferation</td>
</tr>
<tr>
<td>RPE</td>
<td>Retinal pigment epithelium</td>
</tr>
<tr>
<td>FOCUS</td>
<td>RhuFab V2 Ocular Treatment Combining the Use of Visudyne to Evaluate Safety</td>
</tr>
<tr>
<td>SAILOR</td>
<td>Safety Assessment of Intravitreous Lucentis for AMD</td>
</tr>
<tr>
<td>SERS</td>
<td>Second eye ranibizumab study</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious adverse events</td>
</tr>
<tr>
<td>SD-OCT</td>
<td>Spectral-domain optical coherence tomography</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical package for the social sciences</td>
</tr>
<tr>
<td>SRF</td>
<td>Sub-retinal fluid</td>
</tr>
<tr>
<td>SRH</td>
<td>Sub-retinal haemorrhage</td>
</tr>
<tr>
<td>SRC</td>
<td>Sydney retina clinic</td>
</tr>
<tr>
<td>TaE</td>
<td>Treat and Extend</td>
</tr>
<tr>
<td>TD-OCT</td>
<td>Time-domain optical coherence tomography</td>
</tr>
<tr>
<td>TAP</td>
<td>Treatment of age-related macular degeneration with photodynamic therapy study</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>USD</td>
<td>United States Dollars</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>VIEW</td>
<td>VEGF trap-eye: Investigation of efficacy and safety in wet AMD study</td>
</tr>
<tr>
<td>VIP</td>
<td>Verteporfin in photodynamic therapy study</td>
</tr>
<tr>
<td>VA</td>
<td>Visual acuity</td>
</tr>
</tbody>
</table>
PUBLICATIONS ARISING FROM THIS THESIS


*Chew JK, *Zhu M, Broadhead GK, Luo K, Hong T, Chang A. Bilateral neovascular age-related macular degeneration: Comparisons of clinical presentation and outcomes between first and second eyes. Ophthalmologica – The European Retina Journal (Accepted for publication on 13/2/2017 )

*Zhu M, and Chew JK contributed equally to the publication and share first authorship for both publications.
PUBLISHED ABSTRACTS FROM THIS THESIS


ABSTRACT

**Background:** Intravitreal anti-vascular endothelial growth factor (anti-VEGF) agents are the established standard of care for neovascular age related macular degeneration (nAMD), however there are currently limited data on long-term outcomes of this therapy. Ranibizumab is one such anti-VEGF agent administered to treat nAMD.

Patients diagnosed with nAMD undergo regular clinic based follow-up as part of their treatment, often on a monthly basis. Assessment during these appointments includes optical coherence tomography (OCT) scans, which can contribute to the detection of nAMD in the second eye. There is limited data on the symptomatic status, clinical presentation and outcomes of second eye nAMD whilst undergoing regular assessment for the first treatment eye under these conditions.

**Aims:**

The first aim of this thesis is to evaluate the long-term (5-year) outcomes of intravitreal ranibizumab (an anti-VEGF agent) in treating nAMD by examining a cohort within a real life clinic setting. The second aim is to compare the clinical presentation and treatment outcomes between the first and second treated eyes in patients that developed nAMD in both eyes, whilst under regular review for unilateral nAMD.

**Methods:** A total of 208 patients (208 eyes) were included in a retrospective case series assessing the 5-year outcomes of nAMD treated with ranibizumab, entitled the long-term ranibizumab study (LTRS) (Chapter 3). Intervention was an individualised treatment model after three initial monthly loading doses. Visual acuity (VA), central macular thickness (CMT), qualitative OCT features, and adverse events (AE) were determined for each visit. Snellen VA was converted to Early Treatment Diabetic Retinopathy Study (ETDRS) letters for analysis.
To assess outcomes of second eyes diagnosed with nAMD, a retrospective case series entitled second-eye ranibizumab study (SERS) forms the second part of this thesis (Chapter 4). Forty-five consecutive patients fulfilled the inclusion criteria of commencing treatment with ranibizumab in the first eye for nAMD between July 2007 and March 2011, and subsequently developing nAMD in the second eye with at least 12-months of follow-up in each eye. Treatment was administered under the same conditions as the LTRS. Snellen VA was measured, and OCT examination of both eyes at each visit assessed the presence of intra-retinal fluid (IRF) and sub-retinal fluid (SRF). Patient reported symptoms were recorded at every clinic visit.

Paired t-tests were used to assess changes in VA and CMT over the study duration of the LTRS and SERS and two sample t-tests were used to evaluate VA differences between groups. Changes in VA compared to baseline were classified into the three categories: stable VA (loss or gain of ≤15 letters), improved VA (gain of >15 letters), or worse VA (loss of >15 letters). Linear regression was used to assess the effects of age, gender, number of injections, previous treatment, medical history, medications, and baseline VA on both VA and CMT changes. Chi-square test or Fisher’s exact test were used to measure proportions of patients with visual stability and OCT fluid free status at 12-months in the SERS.

**Results:** In the LTRS, mean VA improved by 1.9 letters after 1 year (p=0.020) and decreased by 2.4 letters over 5-years of the treatment (p=0.040). At the end of year 5, 11.1% (23/208) of patients improved VA by more than 15 letters and 68.8% (143/208) of patients had stable VA, while 20.2% (42/208) patients lost more than 15 letters. Patients with VA less than 35 letters (approximate Snellen VA 6/60) at baseline showed significant VA improvement after 5-years of treatment (mean increase 11.5 letters, p=0.01), whilst those that were between 70 and 85 letters (approximate Snellen VA 6/12 to 6/6) at baseline showed a mean decrease (-12.9 letters, p=<0.001). There was a positive relationship between injection numbers and
VA improvement over the 5-years after adjusting for age and baseline VA (Regression coefficient 0.3, P<0.001). Mean CMT decreased by 28.3µm (p<0.01) over 5-years. Ocular AE, ocular serious adverse events (SAE) and systemic SAE occurred in 4.6%, 0.48% and 2%, respectively, during the follow-up period. Thirty-six per cent of patients did not require an injection in their 5th year of follow-up, whilst 24% of patients required near monthly (10-12) injections in the 5th year.

In the SERS, second treated eyes commencing treatment with good VA at baseline (defined as >76 letters, or Snellen VA approximately 6/9)) showed greater stability of vision at 12-months vs. first treated eyes (p=0.05). There was no significant difference in mean VA change between first and second treated eyes. The proportion of OCT - fluid free eyes was higher amongst second treated eyes compared with first treated eyes at 12-months (70% vs. 40%, p=0.02). Intra-retinal fluid (IRF) was seen in 54% of second treated eyes at baseline compared with 84% in first treated eyes (p=0.01). Symptoms were absent in 54% of second treated eyes at baseline. The most common symptoms were “blurred vision” (28% of all patients) and metamorphopsia (11% of all patients).

**Conclusions:** The visual gains achieved were not as significant as clinical trials, likely reflecting the differences in inclusion criteria of patients, and less rigorous follow-up and treatment. Intravitreal ranibizumab was effective in maintaining vision in patients with nAMD and reducing macula thickness over 5-years using an individualised treatment regime in a real-world setting. Ranibizumab is a safe drug to use over 5-years in a real-world clinical setting.

In patients undergoing treatment for nAMD in the first eye, OCT screening of the second eye at each visit may be necessary to detect second eye nAMD in this at-risk population. A large proportion of patients are asymptomatic at diagnosis of second eye disease, and a significant proportion of patients were detected to have treatable subfoveal nAMD with OCT alone. Second eye disease detected and treated by such
a protocol showed a lower rate of IRF at baseline, suggesting early detection had occurred. Second eyes showed a higher rate of fluid free status at 12-months compared to the first treated eye, suggesting that early detection and treatment led to improved anatomical outcomes, potentially explaining superior VA outcomes. Patients commencing treatment in their second eye with good VA had better visual outcomes compared to those with worse VA.
CHAPTER ONE - Literature review

1.1 - Introduction

Neovascular age-related macular degeneration (nAMD, also known as exudative AMD) is the commonest cause of visual impairment in people over the age of 50 in the developed world. (1) Visual loss causes significant functional impairment (including deficiencies in reading and fine motor tasks) and is a risk factor for serious co-morbidities, conferring an increased risk of falls and subsequent hip fractures. (2-4)

Ranibizumab, (Lucentis, Genentech Inc. South San Francisco, CA) an anti-vascular endothelial growth factor (anti-VEGF) inhibitor is an effective treatment to reverse and stabilise visual loss secondary to nAMD. Several studies have shown that, untreated nAMD can lead to legal blindness in the affected eye within 3 years. (5) Since ranibizumab was introduced in 2006, it has revolutionised the treatment of nAMD. In Australia, ranibizumab has reduced incident blindness from newly diagnosed nAMD over a 2-year period by an estimated 72%. (6)
1.2 - Definition of age-related macular degeneration:

Age-related macular degeneration (AMD) is a common, progressive bilateral degenerative condition of the central part of the retina (known as the macula) that affects older persons, resulting in a wide spectrum of central visual impairment. The macula allows patients to read and observe subtle changes in their environment (Figure 1). Bilateral macular function is important for depth perception and hence critical for fine motor tasks.
Figure 1 – Schematic diagram of macular degeneration - This figure is taken from Arroyo JG. A 76-Year Old with Macular Degeneration. JAMA 2006; 295:2394.
1.3 - Classification of age-related macular degeneration

Several methods of classification have been described for AMD, with epidemiological data reported utilising variations of each classification system. The following classification is based on the presence or absence of a choroidal neovascular membrane (CNVM).

1.3.1 - Dry age-related macular degeneration

“Dry” (non-exudative, or non-neovascular) – comprises approximately 85% of diagnosed disease. (7) This encompasses a spectrum ranging from small drusen (asymptomatic to mild visual loss) to advanced geographic atrophy (Figure 2) involving the fovea (generally with resultant Snellen visual acuity (VA) <6/60, classified as “legal blindness” in Australia).

Drusen are localised deposits of extracellular material (mainly lipofuscin) lying between the RPE and the inner collagenous zone of Bruch’s membrane (7, 8) resulting from photoreceptor metabolism. In AMD, drusen is predominantly concentrated at the macula. (9). The term “drusen” is derived from the German word for geodes; cavities in rocks often lined by crystals

In geographic atrophy (a manifestation of late stage AMD) Bruch’s membrane is markedly disrupted, with prominent RPE and photoreceptor atrophy (Figure 3) in addition to loss of inner retinal cell types including amacrine, horizontal and bipolar cells.
Figure 2 - Fundus photography of “dry” or non-exudative age-related macular degeneration: Panel A shows early disease (fine drusen), Panel B shows moderate disease (soft, coalescing drusen) and Panel C shows late disease (geographic atrophy)
1.3.2 – Neovascular age-related macular degeneration

The neovascular type of AMD (also known as exudative, or wet AMD) accounts for 15% of all AMD, yet is responsible for 90% of AMD associated blindness. (10) Angiogenesis is the process of new vessel formation sprouting from existing vessels. In the normal physiology of the eye, angiogenesis is important for tissue development, growth and repair. Pathological angiogenesis is the hallmark of nAMD. This occurs in the form of a CNVM (an ingrowth of new vessels from the choriocapillaris through Bruch’s membrane (the innermost layer of the choroid), fibrovascular pigment epithelial detachment (PED), an area of retinal angiomatous proliferation, (RAP resulting from growth from the deep capillary plexus of the retina growing downwards towards the RPE) or polypoidal choroidal vasculopathy (PCV, a branching choroidal network characterised by a bulb and stalk configuration of vessels with surrounding dilatation of the choroidal vessels.) The result is damage to the photoreceptors by collections of fluid or blood leaked from these abnormal vessels. Patients may also present with a disciform scar that indicates a previously active CNV (Figure 4, panel C)

In contrast with non-exudative AMD, nAMD causes more severe and rapid vision loss and treatment has the potential to restore vision.
Figure 3 - Fundus photography and fluorescein angiography showing enlarging areas of RPE atrophy over a 2-year period, representing progressive geographic atrophy
Figure 4 - Fundus images illustrating soft drusen (A), choroidal neovascular membrane with sub-retinal haemorrhage (B) and resultant disciform scar (C).
1.3.2 - Grading the severity of age-related macular degeneration

Severity of AMD may be graded based on clinical appearance of the macula with funduscopic examination or fundus photography (Table 1). In 2013 a panel of 26 AMD experts published a consensus-derived clinical classification system using a modified Delphi process. (11) The fundus signs were based on a location of within 2 disc diameters of the fovea in persons >55-years old. (11)

Table 1 – Age-related macular degeneration severity and key features

<table>
<thead>
<tr>
<th>AMD Severity</th>
<th>Key Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>No AMD</td>
<td>The absence of visible drusen or pigmentary abnormalities</td>
</tr>
<tr>
<td>Normal Aging Changes</td>
<td>The presence of small drusen only (also known as “drupelets” &lt;63 μm in size)</td>
</tr>
<tr>
<td>Early AMD</td>
<td>The presence of medium drusen (&gt;63μm and &lt;125μm, correlating to the width of a retinal vein when exiting the optic disc) with an absence of pigment abnormalities.</td>
</tr>
<tr>
<td>Intermediate AMD</td>
<td>Large drusen (&gt;125μm) or at least medium drusen associated with RPE abnormalities (hyperpigmentation or hypopigmentation)</td>
</tr>
<tr>
<td>Late AMD</td>
<td>Presence of neovascular AMD or geographic atrophy</td>
</tr>
</tbody>
</table>
The utilisation of this classification system in conjunction with the findings of the age-related eye disease study (AREDS) aids prognostication of the 5-year risk of progression to late AMD. (12) This clinical tool helps to guide the physician on the need for more rigorous follow-up should the patient present in a high-risk category (Figure 11). It also aids in identifying a group of patients who were proven to derive the most benefit from vitamin supplementation, with the endpoint of decreasing the risk of progression to more advanced AMD. (13)
1.3.3 - Classification of neovascular age-related macular degeneration

The method of anatomical nAMD classification was described by Gass et al. (14) and is based on the lesion characteristics as seen with fundus fluorescein angiography (FFA) characteristics and/or indocyanine green angiography (ICG). ICG is an alternative means of imaging the ocular circulation, with increased resolution of the choroidal circulation. The terminology is outlined in Figure 5, with the categories being “occult”, “classic” and “RAP”. These descriptive terms are based on the filling patterns of the fluorescein within the neovascular lesion, which correspond to the anatomical position of these vessels in relation to the RPE layer. In Australia, a FFA demonstrating subfoveal CNV secondary to AMD in patients >50 years old is required to access government subsidised anti-VEGF agents, namely ranibizumab, and aflibercept.

Prior to the availability of modern anti-VEGF agents, the distinction between “occult” and “classic” lesions (described below) was important to guide treatment with verteporfin photodynamic therapy (PDT). The Verteporfin in photodynamic therapy study (VIP) and Treatment of Age-Related Macular Degeneration with Photodynamic Therapy (TAP) studies showed that PDT was only effective in CNV with a predominantly “classic” component. (15, 16) In Australia, this is reflected by the pharmaceutical benefits scheme (PBS) funding approval criteria, where access to PDT is only permissible if the lesion is “shown by FFA to be subfoveal, and have a predominantly (>50%) classic appearance, in addition to a baseline VA of ≥6/60.” The Minimally Classic/Occult trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular Age-Related Macular Degeneration (MARINA) (17) and Anti-VEGF antibody for the treatment of predominantly classic choroidal neovascularisation in AMD (ANCHOR) (18) trials demonstrated that both classic and occult lesions responded well to ranibizumab, hence there is no such requirement
stipulating angiographic lesion type and visual acuity for access to ranibizumab in Australia.
Figure 5 - Classification of choroidal neovascularisation based on angiographic appearance
1.3.3.1 - Type 1 Choroidal neovascularisation (occult)

The CNV is located beneath the RPE, and is termed “occult” as there is no clear leakage source identified on FFA. There is ill-defined early hyperfluorescence, (due to blockage of fluorescein by the RPE, meaning that more fluorescein dye needs to accumulate before a lesion is apparent) with late stippled hyperfluorescence seen on FFA with indistinct margins, representing late leakage of fluorescein.

The majority of CNV in nAMD is of the occult subtype, which is sub-categorised into fibrovascular pigment epithelial detachments (PED’s) (type 1 occult lesion, Figure 6) and late leakage of undetermined source (type 2 occult lesion).
Figure 6 - Occult Choroidal neovascular membrane (Type 1 choroidal neovascularisation) imaging – fundus photo (A), early (B), mid (C) and late (D) phase fluorescein angiography, demonstrating an increasing area of diffuse hyperfluorescence, indicating diffuse leakage. Corresponding optical coherence tomography (OCT) illustrating sub-retinal fluid (arrow, E)
1.3.3.2 - Type 2 choroidal neovascularisation (classic)

In this subtype, the CNVM has penetrated the RPE and lies above it (in the sub-retinal space) resulting in early hyperfluorescence on FFA that progressively increases in size and intensity during the study. The margins of the lesion are well defined in the later phase of the angiogram (Figure 7). Classic lesions typically lead to a more rapid deterioration of vision compared to occult lesions (18), hence their identification is important to ensure treatment is commenced promptly. Furthermore, this subtype was shown to be more responsive to previous treatment modalities including PDT. (15, 16)
Figure 7 - Classic choroidal neovascularisation (Type 2 choroidal neovascularisation) imaging: fundus photo (A), early (B), mid (C) and late phase FFA (D), showing early hyperfluorescence with well demarcated borders, and SD-OCT illustrating sub-retinal fluid (E, arrow).
1.3.3.3 - Type 3 choroidal neovascularisation: (Retinal angiomatous proliferation)

This type of neovascularisation originates from the retinal circulation, limited to the neurosensory retina in early stages, and merging with the choroidal circulation in advanced disease. (19) It is best demonstrated using ICG angiography, however can be visualised with FFA (Figure 8). The term “Type 3 CNV” was popularised by Freund in 2008. (20) The incidence of RAP lesions is influenced by ethnic background, with a much lower rate of this CNV subtype in Asians compared to Caucasian populations. (21)
Figure 8 – Retinal angiomatous proliferation lesion (Type 3 choroidal neovascularisation) - fundus photo showing intraretinal haemorrhage at the fovea (A), fundus fluorescein angiography shows diffuse leakage (B-C), and optical coherence tomography shows intra-retinal cysts (D).
1.3.3.4 - Polypoidal Choroidal Vasculopathy

Polypoidal choroidal vasculopathy (PCV) is an additional subtype of nAMD that is often included in the classification of nAMD, and is more prevalent in Asia. Despite sharing some common genetic traits with AMD, specifically the LOC3877715/HTRA1 variant (22) several authors regard this condition as a separate entity, evidenced by its markedly different response to PDT and anti-VEGF agents. (23) (24) In contrast, many authorities have subsequently considered it to be a form of nAMD (25). This lesion is characterised by a branching choroidal network with surrounding polypoidal (resembling a small growth, with a stalk) dilatation of the choroidal vessels, leading to recurrent serous leakage and haemorrhage. (26) The lesion is best visualised with ICG angiography. (23) Polypoidal choroidal vasculopathy must be considered in the differential diagnosis when treating a patient of Asian descent, evidenced by a recent study by Maruko et al. which found a 54.7% prevalence of PCV in eyes of Japanese patients who had been diagnosed with AMD, (27) highlighting the likelihood of widespread under-diagnosis of this condition.
1.4 Epidemiology

1.4.1 - The global impact of macular degeneration

Age-related macular degeneration is the leading cause of blindness in western countries for those aged over 55, eclipsing other blinding conditions including glaucoma, diabetic retinopathy and cataract. (28-30) It is estimated to affect 30 – 50 million individuals worldwide. (31-35) In the USA approximately 15 million people have “dry” AMD whilst 1.7 million people have nAMD, representing 10-15% of all AMD. The estimated incidence of new cases of exudative AMD in the USA is 200,000, where it is the leading cause of blindness, with an expected two-fold increase in prevalence by 2020. (32, 36)

Comparisons of the scope of patients’ affected by this disease can be made with cancer (2007 prevalence of 12 million in the US) (37) and Alzheimer’s disease (2007 prevalence of 5 million in the US). (38) In the UK, prevalence of nAMD in those aged 50 years or more was 10% in 2012. (39) UK annual incidence of nAMD was 2.3 per 1000 in women, and 1.4 per 1000 in men, totalling 3.7 per 1000 persons in 2012. (39)
1.4.2 - Macular degeneration in Australia:

Consistent with much of the developed world, macular degeneration is the leading cause of blindness in Australia, causing 50% of all legal blindness. (40)

Prevalence of age-related macular degeneration:
An estimated 1 million people over 50 (14.3% of Australians over 50) have AMD, with the numbers of each subtype shown in Figure 9. The prevalence of AMD is 4 times greater than that of dementia, and half that of diabetes, (41) and therefore a major public health concern.

Incidence and Prevalence of neovascular age-related macular degeneration:
In 2010, 110 000 Australian patients had nAMD. There were 7622 new cases diagnosed in 2014 leading to treatment with anti-VEGF agents of ranibizumab or aflibercept (Department of Human Services Medicare Pharmacy Claims database, accessed April 2015) giving an incidence rate of 0.036% or 3.6 per 1000 persons. The number of new cases of nAMD in Australia has remained reasonably stable at 7000 to 8000 cases between 2007 and 2014. (Table 1)

Treatment frequency and duration:
Recent prevalence data from the Department of Human Services reports that in 2014, 36,739 patients received at least one intra-vitreal injection of an approved agent for nAMD (ranibizumab or aflibercept) and 249,722 injections were dispensed in total (Table 1). Approximately 3000 patients are into their seventh year of treatment (39% of patients initiated with therapy in 2007) indicating that ongoing treatment is required for many patients, and therefore the long-term efficacy and safety is of importance. This will be addressed in the long-term ranibizumab study (LTRS).
Projected rates:

Age-related macular degeneration prevalence rate in Australia is projected to increase to 1,700,000 by 2030 driven by an aging population. (41)
Figure 9 - 2010 estimated prevalence of age-related macular degeneration in Australia in those age > 50 years. For these statistics, "dry AMD" refers to late, atrophic stage (Geographic Atrophy) with a visual acuity of 6/12 or worse.
Table 2 – Summary of age-related macular degeneration treatment in Australia, and utilisation of ranibizumab and aflibercept. *The figure for 2007 represents the first five months of listing. Source: Department of Human Services Medicare Pharmacy Claims database, accessed April 2015

<table>
<thead>
<tr>
<th>Year</th>
<th>New patients</th>
<th>Prevalent patients</th>
<th>Prescriptions supplied</th>
<th>Injections supplied</th>
<th>Calculated injections per prevalent patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007*</td>
<td>7,478</td>
<td>7,478</td>
<td>17,482</td>
<td>18,492</td>
<td>2.47</td>
</tr>
<tr>
<td>2008</td>
<td>8,205</td>
<td>14,320</td>
<td>59,786</td>
<td>63,177</td>
<td>4.41</td>
</tr>
<tr>
<td>2009</td>
<td>8,125</td>
<td>18,889</td>
<td>93,087</td>
<td>98,612</td>
<td>5.22</td>
</tr>
<tr>
<td>2010</td>
<td>7,654</td>
<td>22,834</td>
<td>130,814</td>
<td>140,245</td>
<td>6.14</td>
</tr>
<tr>
<td>2011</td>
<td>7,097</td>
<td>26,132</td>
<td>160,194</td>
<td>172,548</td>
<td>6.6</td>
</tr>
<tr>
<td>2012</td>
<td>6,874</td>
<td>29,269</td>
<td>188,027</td>
<td>203,751</td>
<td>6.96</td>
</tr>
<tr>
<td>2013</td>
<td>7,765</td>
<td>33,347</td>
<td>208,919</td>
<td>227,193</td>
<td>6.81</td>
</tr>
<tr>
<td>2014</td>
<td>7,622</td>
<td>36,739</td>
<td>229,083</td>
<td>249,772</td>
<td>6.8</td>
</tr>
</tbody>
</table>
1.4.3 - Incidence of second eye neovascular age-related macular degeneration

There is a large reported range of incidence rates for nAMD being diagnosed in the second eye. A meta-analysis of 53 studies (including 28 randomised trials) by Wong et.al. found a rate of 12.2% at 12-months and 26.8% by 48 months. (5) Epidemiology studies have reported incidence rates of second eye nAMD in patients with unilateral nAMD ranging between approximately 12% and 22% at 2 years. (42) Five-year retrospective analysis of second eyes in the macular photocoagulation study (MPS) without CNV at baseline showed a 25.7% incidence of CNV at 5-years, therefore an extrapolated annual incidence rate of approximately 6% per year was noted, whereas the sub-macular surgery trials reported a CNV rate of 12.8% per year in second eyes. (43) (44) MARINA and ANCHOR trials reported 2-year incidence rates between 23.8% and 36.3% in both treatment and control arms, (42) Although this is the highest rate reported, it is likely the most accurate; reflecting earlier and more accurate detection of nAMD due to the rigorous and regular assessments including the use of optical coherence tomography (OCT) mandated by a trial protocol.

There is a suggestion of ethnic variation in the incidence of second eye CNV, with AREDS prospective 5-year data reporting a 30.8% rate in a Caucasian population, (45) compared to 12% in a Japanese population. (46) This lower incidence may be partially related to differences in the complement factor-H genotypes of Japanese patients. (47)
1.4.4 - Timing of neovascular age-related macular degeneration developing in the second eye relative to the first eye

Gudnadottir et.al (48) reported a subset of 65 patients (from a cohort of 2220 individuals, with a maximum follow-up duration of 13 years) who developed second eye nAMD following diagnosis in the first eye. They found a mean time interval of 2.5 year between eyes, and a median time of 1.8 years.
1.5 - Population screening for age-related macular degeneration

Utility of the Amsler Grid as a diagnostic tool:

A “screening test” can be defined as a population based strategy to target a test towards an “at risk population” that has a high positive predictive value. Although there is no proven screening population based screening program for AMD, some public health policies have promoted the use of an Amsler grid as a screening tool for all patients over the age of 50.

The purpose of the Amsler Grid is to detect new metamorphopsia or scotoma. (49) The patient is asked to gaze at the centre of the grid as frequently as possible. (Figure 10) Its advantages include minimal cost of production, ease and speed of administration, and the ability of the test to be self-administered by the patient at home.

Schlaegel et.al. in 1968 (50) was one of the first advocates of using an Amsler grid to monitor central vision with the aim of detecting macular disease, and thus it is a practice over 40 years old. Its ability to detect visual disease has remained doubtful in several studies (51) and many clinicians feel that it is not a sufficiently sensitive instrument for monitoring vision. (52, 53) Reasons for its shortcomings include likely non-compliance by patients (54) and physiological adaptation by the brain in the form of a “cortical filling in” mechanism leading to a failure to perceive small lesions. (55) Despite its limitations, the Amsler grid remains the most commonly used home tool for screening for the incidence and progression of macular disease, and patients are routinely asked to present for medical care should they notice a change in their vision using this tool.
Figure 10 - Amsler Grid - Left panel shows the grid viewed without macular disease, whilst the right panel illustrate a paracentral scotoma and metamorphopsia seen in macular degeneration.
1.6 - Ocular risk factors for neovascular age-related macular degeneration:

1.6.1 - Ocular signs of age-related macular degeneration observed on clinical examination:

The presence of large drusen (>125um) and pigmentary changes on funduscopic examination or fundus photography have been identified as risk factors for developing advanced AMD in the AREDS study. (13) This culminated in a severity assessment scale, which is designed for use by a clinician to quickly assess the 5-year risk of developing advanced AMD (Figure 11). (12)

Advanced AMD (either nAMD or geographic atrophy) that presents in one eye has been shown to be a strong predictor of progression to advanced AMD in the remaining eye without AMD (8.6% in eyes with advanced AMD in the second eye vs. 0.7% in eyes without advanced AMD in the second eye at 10-years following baseline) (56) This increased rate of developing nAMD is also seen in eyes with early AMD changes, such as small to intermediate size drusen, and RPE abnormalities, where the second eye has advanced AMD. Up to 76% of such eyes will develop nAMD after 10-years (56). Both these findings suggest that the presence of nAMD in one eye will likely result in the same disease process in the remaining eye, and is a reminder that nAMD can be considered a bilateral process.
Figure 11 - Algorithm for stratifying risk of progressing to late age-related macular degeneration at 5-years, based on funduscopic signs. (12) Taken from Ferris et.al, 2005 – A simplified severity scale for age-related macular degeneration: AREDS Report No.18.
1.7 Natural history of neovascular age-related macular degeneration:

Progression of early age-related macular degeneration to neovascular age-related macular degeneration:
Chew et al. (56) examined long-term (10-year) outcomes of 3549 participants and addressed the natural history of patients who present with early AMD. (56) Those treated with the AREDS vitamin supplementation formula were found to have a lower rate of CNV, but not GA at 10 years.

Consistent with the findings from the AREDS Report No.18, (12) patients classified in the most severe group of AMD had high rates of progression to advanced AMD by 10 years (48.1% for nAMD and 26.0% for GA.) There was a positive correlation between the severity of AMD at presentation and risk of late AMD at 5-years.

Progression of neovascular age-related macular degeneration:
Prior to modern effective treatments for nAMD, several prospective natural history studies were ethically permissible, including the placebo arm for the MARINA trial for occult sub-foveal CNV, a pivotal study establishing anti-VEGF therapy as the modern standard of care. (17) Once nAMD has developed, the natural history of the disease without treatment leads to legal blindness.
1.7.1 - Rate of visual loss:

Wong et al. published a comprehensive meta-analysis examining the natural history of untreated nAMD from 53 interventional studies, including 28 randomised clinical trials. The average VA loss was 1 line of logMAR VA at 3 months, increasing to 4 lines at 24 months compared to baseline. The proportion of patients with VA deterioration compared to baseline was 45% at 3 months, and 81.1% at 2 years. Legal blindness (defined as logMAR VA >1.0 or Snellen VA <6/60), was noted in 19.7% of patients at baseline 50.3% at 3 months and 77.6% at 3 years. (5) There was great variation in the blindness rates between studies within the meta-analysis.

In contrast, Bressler et al. reported outcomes from 103,582 patients with nAMD, and found a much lower rate of legal blindness at 16% within 2 years of diagnosis without treatment. (57) The data must be interpreted with caution, as some patients were included in the study at a variable time since their onset of disease, therefore pre-existing visual loss due to nAMD, prior to study inclusion may not be included. Despite this caveat, these findings highlights the severe and swift functional deficit that results from untreated exudative AMD, and provide a means of extrapolating long-term visual outcomes of untreated disease.

Establishing the rate of vision loss is important, as the timing of initiating anti-VEGF therapy with nAMD for a patient with good VA (e.g. 6/6 Snellen) remains controversial. An argument to treat early is to cause CNV regression before permanent retinal structural damage occurs, minimising irreversible visual loss. An argument against treatment surrounds the potentially unnecessary exposure to the risk of developing iatrogenic endophthalmitis and other ocular serious adverse events (SAE) (discussed in 1.14 - Safety concerns of anti-VEGF agents and its administration).
1.7.2 - Risk of blindness after reporting symptoms

Information regarding patients’ progression following symptoms for nAMD is scarce. Walsh et.al. reported the proportion of patients progressing to legal blindness (Snellen VA <6/60) following symptomatic presentation of foveal involving nAMD. He reviewed 476 patients and related the length of symptoms vs. the proportion of patients who were legally blind. (58) Walsh’s data suggests that visual deterioration occurred rapidly after nAMD symptoms were noticed, with 32% of eyes legally blind after 1 month of symptoms and 45% after 2 months of symptoms. (Figure 12) Therefore, it follows that early detection and treatment of both symptomatic and asymptomatic disease would improve visual outcomes. Data regarding progression of asymptomatic disease is lacking.
Figure 12 - Correlation of duration of symptomatic neovascular age-related macular degeneration related visual loss and percentage of cases with visual acuity less than 6/60 Adapted from Walsh et.al (58)
1.8 - Clinical presentation of age-related macular degeneration

In patients >50 years of age, several small hard drusen (<63um diameter) are often a normal macular finding and represent age-related changes. (59) These do not affect subjective vision, unlike "soft" drusen that can affect vision. Soft drusen and RPE changes are signs of moderate macular degeneration and are both risk factors for progression to late AMD. (12)

Patients may present for care at various stages of disease ranging from asymptomatic drusen at the macula to massive sub-foveal haemorrhage from nAMD causing acutely impaired vision. Furthermore, they may initially present with early and intermediate AMD, and progress to nAMD without any noticeable visual changes. (60) There is debate as to whether AMD pathogenesis involves linear progression from early stages to the late AMD stages. Neovascular AMD and GA may represent two distinct diseases with distinct biochemical pathways, rather than representing the end-stage sequelae of the same condition. (61)

1.8.1 - Symptoms of neovascular age-related macular degeneration:

There are a range of presenting symptoms for nAMD, including a central scotoma (Figure 13), or distortion of central vision (metamorphopsia, often expressed as a warping or distortion of straight lines (Figure 10). Increased glare, and decreased or asymmetric contrast sensitivity has been described. Micropsia (minification of objects) can occur, as can floaters or generalised blurring of the visual field (62). There are few studies reporting symptoms in macular disease, however blurred vision and distortion were the most commonly reported first symptoms of a macular CNV in a study by Fine et.al. (54) Furthermore, there is limited published data on the symptoms of early nAMD.
Presentation for ophthalmic assessment can be delayed in patients, who either do not notice early changes in their vision, or attribute them to other ocular conditions such as cataracts, deferring assessment. (58) There is little data describing the outcomes in these asymptomatic patients who may have treatable sub-clinical nAMD lesions.
Figure 13 – Neovascular age-related macular degeneration patient with simulated loss of vision with a central scotoma.
1.8.2 - Signs of neovascular age-related macular degeneration

A disciform scar (Figure 4, right panel) represents the endpoint of a previously active CNV with retinal haemorrhage. Visible haemorrhage in the posterior pole is only found in a small proportion of patients (Figure 14). (31)

The presence of “soft drusen” at the macula is a prominent finding in those with AMD, and is a risk factor for developing nAMD in a Caucasian population (Figure 15). (45) A distinct lack of soft drusen has been noted in Asian patients who develop CNV, evidenced by the findings of Uyama et.al. who reported on precursor signs of second eye nAMD in a Japanese cohort. Serous PED was found to be the most common predisposing lesion for nAMD (58%) with soft drusen second (18%). (46) Further discussion on the imaging features of nAMD is provided in subsection 1.10.
Figure 14 - Macular haemorrhage secondary to neovascular age-related macular degeneration seen with fundus photography
Figure 15 – Regressing calcified drusen and pigmentary changes at the macula
1.9 - Diagnosis of neovascular age-related macular degeneration

For many years the gold standard for diagnosing and monitoring CNV in nAMD has been FFA, which is usually conducted following a funduscopic examination from an ophthalmologist showing signs suggestive of active nAMD. A FFA is also indicated if a patient reports new visual symptoms suggestive of macular pathology, including metamorphopsia, difficulty reading, or generalised blurring of vision. This latter practice was based on recommendations from the MPS study in 1993, (44) and remains largely unchanged in recently published guidelines by the European Society of Retina Specialists (EURETINA) in 2014. (63)

Fundus fluorescein angiography is a time consuming, invasive investigation, with a 1:222000 risk of potentially fatal anaphylaxis (64), all factors driving the search for an alternative imaging modality.

Furthermore, limitations with FFA’s ability to detect early CNV have been reported. This is evidenced by histopathological analysis of resected CNV membranes that contained fenestrated sub retinal vessels consistent with CNV, without demonstrating leakage on angiography. (65) Additionally, the size of surgically removed CNV membranes have been found to be 50% larger than their measured angiographic size, suggesting that a small CNV could remain angiographically silent. (66) FFA remains the preferred modality for diagnosis of nAMD, however progress may be effectively monitored by OCT. (67)

There is recent interest and research in OCT angiography (OCT-A), which provides a non-invasive means to provide a three-dimensional map of the retinal and choroidal vasculature. It compares the differences between sequential OCT scans taken at the same site to construct a map of blood flow at a fixed point in time. As such, OCT-A cannot show leakage of fluid like a FFA, however it may more clearly delineate the size of a CNV, especially in Type 1 CNV compared with FFA. Current limitations
include a small field of view, and increased potential for artefacts compared to FFA. (68) Whilst the technology has not been adopted for widespread clinical use at this stage, this modality shows promise in complementing or displacing FFA as the diagnostic modality of choice for nAMD in the future. Early studies have shown that OCT-A is able to reliably detect Type 1 and Type 2 CNV in nAMD, with a case illustrated in Figure 16. (69)
Figure 16 – Adapted from Moult 2014 – Ultrahigh – speed swept-source OCT-A in exudative age-related macular degeneration: 87 year-old patient with nAMD and active CNV lesion. En face slice of the OCT angiogram at the depth of the choriocapillaris, with the “classic” (yellow) and “occult” (red) components of the CNV outlined, and arrows pointing to movement artefact, (E) Early phase FFA with arrows pointing to the classic lesion. (F) Early phase ICG with arrows pointing to the “occult” CNV. (G) Late phase FFA with arrows pointing at stippled hyperfluorescence. (H) OCT retinal thickness map. (I)
1.9.1 - Fundus fluorescein angiographic findings in neovascular age-related macular degeneration

In addition to describing the pattern of leakage, other characteristics can be observed on FFA to determine the presence of a CNV. The following criteria were used by the reading centre for the MARINA and ANCHOR trials to indicate conversion of the second eye from non-exudative to nAMD: (42) The presence of any of the following characteristics on FFA was considered evidence of nAMD.

- Neovascular or exudative characteristics
- Classic choroidal neovascularisation subtype (Type 2 CNV, Figure 7)
- Fibrovascular PED
- Serous pigment epithelial detachment
- Serous sensory retinal detachment
- Other occult choroidal neovascularisation or late leakage of undetermined source (Figure 8)
- Macular haemorrhage (Figure 14)
- Fibrous tissue
- Photocoagulation scar
1.9.2 - Optical coherence tomography in neovascular age-related macular degeneration

Optical coherence tomography is a non-invasive, imaging modality that utilises interferometry (a technique that measures the reflectance patterns of light, allowing a cross-sectional reconstruction of ocular structures, particularly the retina and choroid). In addition to providing anatomical contours of tissues, it is well suited to the measurement of retinal thickness; a feature that has helped guide management of nAMD. It has been widely adopted since the mid 2000’s in clinical practice, with early devices utilising Time-Domain OCT (TD-OCT). (70) This technique generates reflectance patterns between the tissue of interest and a moving reference mirror, and has been used in the majority of clinical trials involving anti-VEGF agents. Its major limitation relates to the image acquisition time, utilising 768 A-scans, leading to limited image resolution and increased artefacts compared to the next generation of OCT technology, termed Spectral-Domain OCT (SD-OCT) which utilises up to 65536 A-scans. This technical limitation has contributed to the finding that TD-OCT was less sensitive than FFA for detecting new onset nAMD,(71) whereas recent studies have shown that SD-OCT may be more sensitive that FFA, Amsler Grid, or preferential hyperacuity perimetry testing for detecting early nAMD. (71) Secondly, automated segmentation errors of the retinal layers utilising a TD-OCT are well known, especially with severe macular conditions such as nAMD. (69)(72) This issue is further discussed in the methods section, Chapter 2.

Although the resolution of OCT images has improved markedly in the last decade, and there may be a future role for OCT in the diagnosis of nAMD (particularly with OCT-A) FFA is currently the preferred modality for initial diagnosis. OCT is the preferred imaging modality for assessing treatment response via anatomical features
including the presence of IRF, sub-retinal fluid (SRF) and the central macular thickness (CMT) to help guide the ongoing need for anti-VEGF treatment.
1.9.2.1 - Features of neovascular age-related macular degeneration imaged on optical coherence tomography - (73)

1. Fibrovascular pigment epithelial detachment (Figure 17).
   Classically appear as broad elevations of the RPE band, accompanied by serous exudate and/or haemorrhage. Enhanced depth OCT imaging shows solid layers of material with medium reflectivity within the PED, interspaced with hypo-reflective clefts.

2. Serous pigment epithelial detachment (Figure 18).
   Smooth, sharply demarcated, dome shaped RPE elevation on OCT, visible with fundoscopy.

3. Haemorrhagic pigment epithelial detachment
   Similar OCT appearance to serous PED, with marked shadowing of Bruch’s membrane

4. Retinal angiomaous proliferation (Figure 19)
   Classically shows frank cystoid macular oedema overlying a PED, with SRF).

5. Sub-retinal hyper-reflective material / disciform scars
   An amorphous lesion of medium to high reflectivity above the RPE, representing the CNV membrane during a highly vascular initial growth phase. This evolves into a primarily fibrous lesion, leading to disciform scar appearing as a hyper-reflective lesion. Loss or thinning of the overlying photoreceptor layer is also common.

6. Presence of sub-retinal fluid / Serous retinal detachment
   Pockets of hypo-reflective spaces between the RPE and the neurosensory retina. (Figure 7) A homogenous hypo-reflective space is consistent with serous exudate, sparsely hyper-reflective spaces are consistent with fibrin or red blood cells.

7. Presence of intra-retinal fluid
A hallmark event of nAMD is the disruption of the external limiting membrane – photoreceptor complex by invasion and proliferation of the CNV lesion into the retina. This results in leakage of fluid into the neurosensory retina, manifesting as retinal thickening, or cystoid hypo-reflective spaces. (74)
Figure 17 – Fibrovascular pigment epithelial detachment – increased reflectivity underlying broad retinal pigment epithelial detachments.
Figure 18 – Serous pigment epithelial detachment – smooth dome shaped retinal pigment epithelial elevation.
Figure 19 – Retinal angiomatous proliferation – intra-retinal fluid overlying pigment epithelial detachments.
1.9.3 – Indocyanine green angiography

The angiographic features of PCV on FFA can be indistinguishable from occult or classic CNV, (26) highlighting the role of ICG in obtaining a definitive diagnosis. Polypoidal choroidal vasculopathy classical features include exudative and haemorrhagic PED. Up to 85% of eyes with large haemorrhagic and exudative neurosensory detachment and PED are diagnosed with PCV with ICG angiography. Due to the neurosensory detachment component, PCV can also masquerade as central serous chorioretinopathy. Typical ICG angiography findings include a branching vascular network comprising of polypoidal structures and variably sized vascular channels (26) seen in the following case study (Figure 20, Panels C-D). Additionally, polypoidal lesions are typically located at the edge of a PED. A technique of utilising both FFA and ICG angiography in the same testing session can aid in diagnosis and delineating the laser spot size used with therapeutic PDT, Figure 21 illustrates the use of ICG in showing treatment response 3-months later, with no leakage from the polypoidal lesions.
**Figure 20 – Polypoidal choroidal vasculopathy case:** 72 year old male presented with 1 month of left sided visual field loss, VA 6/18 Left eye, diagnosed with IPV. Fundus photo of the affected eye (A). Fluorescein angiography with leakage of fluorescein within the foveal avascular zone, similar in appearance to occult CNV (B). Early phase and late phase ICG angiography at diagnosis showing polyps.(C-D). Optical coherence tomography showing intra-retinal cysts and thickening (E).
Figure 21 – Polypoidal choroidal vasculopathy case 3 months following photodynamic therapy, visual acuity 6/4.8. Late phase indocyanine green angiography pre-treatment (A). Late phase indocyanine green angiography with no leakage seen 3 months post photodynamic therapy (B). Optical coherence tomography showing resolution of the intra-retinal cysts and foveal thickening 3 months post treatment aligned with Figure 16E (C)
1.10 – History of treatment of neovascular age-related macular degeneration

1.10.1 - Thermal laser

Since the 1980’s, this method of destructive photocoagulation was an early treatment modality available for exudative macular degeneration, and remained the only clinically proven means of treatment for almost two decades. The MPS group concluded that the patients with classic, small, extra-foveal or juxta-foveal CNV benefited the most from this treatment. This subgroup represented a small proportion of patients with nAMD and although initial outcomes were superior to observation alone, both groups had poor vision at 5-years of follow-up. (75, 76) An immediate central scotoma would result following laser application, progressively enlarging over subsequent years as a result of laser-induced atrophy that often expands to include the fovea. Other adverse effects included RPE rupture, and sub-retinal or vitreous haemorrhage. (26) The aim of treatment was to arrest the CNV progression resulting in stabilisation of the VA. Improvement of VA did not occur for the majority of patients. Thermal laser is no longer offered to patients for sub-foveal nAMD, as new treatment modalities are more effective at preserving vision.
1.10.2 - Photodynamic therapy

Photodynamic therapy (PDT) was the next major advancement in treatment for nAMD, beginning in the late 1990’s. It was a promising treatment option at the time. Prior to anti-VEGF agents, it was considered to be a treatment option for “classic” or Type 2 CNV membranes, hence early anti-VEGF randomised trails including ANCHOR (77) and RhuFab V2 Ocular Treatment Combining the Use of Visudyne to Evaluate Safety (FOCUS) (78) targeting classic lesions included a control arm utilising PDT.

This treatment modality uses a laser with a wavelength of 689nm to activate Verteporfin (Visudyne; Novartis Pharmaceuticals Corp, East Hanover, NJ) a substance that preferentially adheres to capillary endothelial cells of CNV membranes. A laser pulse of 50 J/cm² intensity, 600mW/cm² for 83 seconds, using a spot size of 1000um more than the largest CNV diameter (measured from FFA) destroys CNV vessels, whilst leaving normal vessels intact. (79)

Studies assessing the efficacy of PDT found that predominantly “classic” or Type 2 neovascular membranes responded best, in patients with visual acuity of 6/60 or better. (15) Additionally, it conferred an increased benefit over thermal laser, in that larger lesions (up to 5400um in diameter) and subfoveal lesions showed some benefit, expanding the potential treatment pool to 30% of all nAMD related CNV. This was a major reason to search for another treatment that could treat a higher percentage of CNV lesions. These criteria are still reflected on the Australian PBS approval application for nAMD treatment. PDT can still be used for “classic” lesions in the rare situation of a patient refusing treatment with anti-VEGF therapy.
1.10.3 - Other treatments less widely adopted

Several surgical treatment modalities for nAMD underwent clinical trials in the past, including excision of the neovascular tissue, (80) subfoveal surgery with cell transplantation, (81) macular translocation, (18-23), radiation treatment (24-27) and trans-pupillary thermal therapy. (28) The role of CNV excision via pars plana vitrectomy vs. laser photocoagulation was explored in the Submacular Surgery Trials, a prospective multicentre clinical trial. (82) Surgery was not found to be superior to laser, possibly related to the collateral damage caused by the surgical trauma to surrounding RPE responsible for the nutritional needs of the macular. (83) The next evolution of treatment to address this issue was to combine CNV extraction with relocating the macular to a bed of undamaged RPE, either by folding the scleral, or performing a 360 degree retinotomy. Some trials showed VA gains in excess of 3 early treatment of diabetic retinopathy study (ETDRS) lines, however many patients experienced post-operative diplopia. (83)

Intravitreal triamcinolone acetonide (IVTA, a corticosteroid) was a promising therapy for nAMD. Conflicting evidence emerged, as animal trials and early clinical reports suggested a benefit of a single injection (84, 85) whilst later studies suggested no difference in visual outcomes when compared to placebo at 12-months. (86) Gillies et.al. did find evidence CNV growth retardation in the first 3 months following IVTA therapy, possibly explained by the bioavailability of IVTA in the eye, however this effect was not maintained at 12-months. (86) IVTA was later combined with PDT where its main benefit was to reduce the amount of verteporfin treatments. (87) Concerns of the adverse events related to IVTA, including increased progression of cataract, and mild to moderate intra-ocular pressure rise (88) also contributed to its position as a second-line treatment for nAMD.
It was clear that existing therapeutic options were of limited benefit, and targeting VEGF directly was the next major advancement in treatment.
1.11 - History of vascular endothelial growth factor and retinal disease

In 1971, Folkman proposed the use of an anti-angiogenic agent to treat ophthalmic disease. (89) One therapeutic target molecule identified was VEGF (a dimeric glycoprotein of approximately 40kDa) (90), which had been associated with ischemia, as evidenced by its presence in hypoxic tumours throughout the body. Its role in mediating neovascularisation in ischemic retinal disorders (diabetic retinopathy and retinal vein occlusions) was demonstrated by Aiello and Adamis in 1994. (91, 92) It has also been implicated in the pathogenesis of retinopathy of prematurity, (93) sickle cell retinopathy, in addition to a secondary role in neovascular glaucoma (94) and inherited retinal dystrophies. (95) In these conditions, the primary stimulus for VEGF up-regulation is reduced oxygen partial pressure, leading to hypoxia in retinal pigment epithelial cells and retinal pericytes (92) resulting in activation of hypoxia inducible factor 1α. (96) This process is unlike nAMD, where the contributing events leading to VEGF up regulation appear to be multifactorial.

Around the same time period, VEGF-A (one of seven VEGF proteins found in mammals along with VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F and placental growth factor (PGF) (90) was identified as having a central role in angiogenesis by several investigators including Folkman in the field of oncology. (97) VEGF-A has at least 9 isoforms, with VEGF-A_{165} being the most common isoform expressed.
1.11.1 Role of vascular endothelial growth factor - physiological

Multiple cells located in the retina produce VEGF, including RPE, vascular endothelial cells, pericytes, Muller cells and astrocytes. This suggests that VEGF plays an important role in ocular homeostasis. (98) Vascular endothelial growth factor has been shown to support neuronal and endothelial cell integrity in the mature retina, (99) leading to endothelial cell survival. (100) Evidence has also shown VEGF signalling plays a key role in maintaining RPE, Bruch’s membrane and choroidal endothelial cells. (90) Therefore, there is concern surrounding its complete inhibition, where its supportive function could be important in slowing down visual loss resulting from the GA phenotype of macular degeneration. (90)

Vascular endothelial growth factor receptors have also been localised to retinal neurons, supporting its role in maintaining neuronal health. (101) (102) Mice rendered unable to express VEGF in the setting of hypoxia developed a motor neuron disease, (103) further adding to concerns of secondary neuronal damage related to VEGF inhibition. (90)

1.11.2 - Role of vascular endothelial growth factor - pathological

Vascular endothelial growth factor is a potent, endothelial cell mitogen, which promotes endothelial cells growth and survival. (90) It is responsible for increased vascular permeability (partially through promoting a fenestrated phenotype) (104) and promoting neovascularisation. Increased VEGF production results from oxidative cell stress, and is usually an effective defensive response of the body to cause proliferation of new blood vessels and a neovascular membrane. These newly formed vessels are immature and leak blood and exudate, damaging surrounding retinal tissues, especially photoreceptors. (79) Clinicopathological studies in humans
have shown increased VEGF-A levels in both the vitreous and excised CNV membranes of patients with nAMD. (105, 106)

Following these discoveries involving VEGF, agents designed to block this protein were developed. The first of these anti-VEGF agents was bevacizumab (Avastin, Genentech) a monoclonal antibody approved for oncological treatment in the United States of America (USA) in 2004 that was adopted for use in the eye. This drug, along with others with a similar mechanism of action (pegaptanib, ranibizumab) binds to endogenous VEGF, hence inactivating the molecule, reducing angiogenesis, and at the time represented the latest revolution in treatment options for AMD. Vascular endothelial growth factor's pathological role was supported by surgically obtained CNVM specimens in patients with AMD, which showed the presence of VEGF-A, leading it to be one of the current major target molecules of therapies for nAMD. (107-109)

Despite identifying VEGF as an end-stage mediator of CNV, a thorough understanding of the primary trigger for nAMD remains incomplete. One proposed theory is that the formation of CNV in nAMD is a secondary reaction to a pathological process that occurred earlier in life. This process may have led to RPE damage, evoking an immune response involving the production of pro-angiogenic factors, upsetting the equilibrium between pro-angiogenic and anti-angiogenic cytokines, thereby promoting CNV. (110)
1.12 Anti-vascular endothelial growth factor agents

Treatment of nAMD has undergone a revolutionary change in the last decade (since 2007) with the standard of care currently being intravitreal anti-VEGF. Each anti-VEGF agent exhibits variations in its exact targets, however their therapeutic endpoint is in reducing the effect of VEGF. Anti-VEGF agents are the first type of treatment that has resulted in the improvement of vision in a large proportion of patients. (18)

The goal of inhibiting VEGF-A is to reduce intra and sub-retinal oedema, by reducing vascular permeability. Anti-VEGF agents can also lead to regression of the neovascular membrane, and should this occur, it is thought to increase the duration of treatment effect. (111) However, improvement of visual function has been found in cases with oedema control without regression of the CNV membrane (MARINA trial), implying that the major driver of visual improvement is the reduction of retinal oedema. (112)
1.12.1 - Pegaptanib

Pegaptanib (Macugen, Eyetech Pharmaceuticals, Pfizer Inc, New York) was the first VEGF inhibitor developed for the eye, and approved by the Food and Drug administration (FDA) of the USA for use in nAMD in December 2004. Unlike ranibizumab, it binds and inactivates only one isoform of VEGF-A (VEGF-A 165).

At 12-months, 70% (206/294) of patients with predominantly “classic” CNV treated with 0.3mg of pegaptanib had stable vision (lost <15 letters) compared with 55% (164/296) of controls treated with sham injections. (p<0.001) (113) This outcome was favourable when compared to PDT.

At 12-months, 6% of patients treated with 0.3mg had an improvement of >15 letters of VA. This rate was far lower than the ANCHOR and MARINA clinical trial cohorts, who were treated with ranibizumab.

During the clinical trials for pegaptanib, concerns were raised surrounding ocular adverse events. There was a 1% endophthalmitis rate, along with similar rates of other serious ocular adverse events including retinal detachments and traumatic cataracts. It is plausible that these events were related to the learning curve surrounding the administration of regular intravitreal injections to large numbers of patients, which at the time was a new practice. (113)

Pegaptanib was not marketed in Australia; hence few patients in this country had access to this treatment apart from those enrolled in clinical trials. In the USA, some early nAMD cases treated with ranibizumab were found to have better outcomes compared to pegaptanib, (79) and consequently it was less used when other anti-VEGF therapies became available. Pegaptanib is no longer recommended as a treatment for nAMD.
1.12.2 - Bevacizumab

Bevacizumab (Avastin, Genentech, San Francisco, California) is a humanised, monoclonal full-length immunoglobulin G (IgG) antibody against VEGF that inhibits the normal function of VEGF-A by binding to all of its isoforms. (114) This was initially developed as an oncology medication, to inhibit pathological angiogenesis in tumours and was in clinical use by 1997. (115) Once approval was given to treat metastatic colorectal cancer by the FDA, its off-label use for ocular conditions, especially nAMD became widespread. Bevacizumab was first reported to be effective for treating nAMD in 2005. (116) It shares the same murine antibody as ranibizumab. (79) Its major structural difference is a larger molecular size at 149-kD, vs. the Fab fragment of ranibizumab at 48kD. This difference results in a longer half-life compared with ranibizumab and likely explains reports of bevacizumab remaining detectable in second eyes after injection, unlike ranibizumab. (117)

Another major difference is its low price at approximately one-fortieth the cost of ranibizumab and aflibercept. Recent head to head studies have demonstrated non-inferiority of bevacizumab when compared to ranibizumab in most treatment regimes. (118, 119)

One of the major concerns of using bevacizumab has been the increased risk of cardiovascular events, stroke and gastrointestinal bleeding. These adverse events have been reported in patients undergoing systemic treatment for oncological indications, (120) and supported by laboratory evidence of the molecule having a prolonged half-life in the systemic circulation. (116-120) The potential safety issues are discussed in Chapter 1.14.
1.12.3 - Ranibizumab

Ranibizumab (Lucentis, Genentech Inc., South San Francisco, CA) was designed as a competitive VEGF inhibitor, originating from murine species. The antibody was available for use in Australian from 2007, and it was the first nAMD treatment that reversed visual loss in a large proportion of patients.

Ranibizumab is a fragment of recombinant, humanised monoclonal antibody targeting multiple isoforms of VEGF-A (VEGF110, VEGF121, VEGF145 VEGF165 VEGF183, VEGF189, VEGF206). These isoforms usually bind to VEGF receptors on the cell surfaces of endothelial and mural cells, leading to endothelial cell proliferation, neovascularisation and increased vascular permeability via a tyrosine kinase-signalling pathway. (79) The absence of the fragment crystallisable (Fc) portion of the AB contributes to its molecular weight of 48kDa, (121) which is smaller than bevacizumab and has been shown to penetrate the retina well. (122) The absence of the Fc portion also removes the possibility of activation of the innate immune system, (123) and leads to rapid systemic clearance, (124) both of which theoretically reducing the risk of systemic adverse events compared to bevacizumab.

It provided a substantial benefit over the 1st generation anti-VEGF treatment introduced in 2004 (pegaptanib) in that it improved or stabilised vision in 90% of patients at 12-months vs. 70% in the pegaptanib arm. (113) Additionally, it proved its effectiveness targeting angiographically classic, occult or mixed lesions unlike PDT, which was only proven to be effective for predominantly classic lesions (a small proportion of nAMD cases).
1.12.3.1 – The evolution of ranibizumab treatment regimes

Since ranibizumab was introduced in Australia in 2007, several studies have influenced the pattern of treatment, specifically impacting on the frequency of injections, and the methods used to determine retreatment.

1.12.3.1.1 - Establishing a clear benefit for ranibizumab

Landmark clinical trials that shifted the paradigm of nAMD treatment towards anti-VEGF agents, and specifically ranibizumab, were the MARINA, (17) and ANCHOR (18) studies. In MARINA and ANCHOR, 90% of patients achieved VA stability (lost fewer than 15 letters) at 24 months. VA gains of 15 or more letters were seen in 33% to 41%. (17, 18) Patients in the treatment arm of the ANCHOR study had a lower rate of legal blindness at the end of the trial (12% vs. 43% of patients in the sham injection group). Therefore head to head studies of ranibizumab against PDT (standard of care for occult lesions at the time of the MARINA study) and sham injections (ANCHOR study) showed that ranibizumab led to superior visual outcomes. Furthermore, the angiographically visible area of CNV lesion showed no significant change over 1 year in the MARINA study, compared with a 2 disc diameter increase in the size in the control group, indicating that ranibizumab arrested the growth of CNVM without leading to immediate regression of the lesion.

A monthly treatment regime was utilised for these studies, which placed great burdens on the patients, their carers, and the health systems, in addition to regularly exposing patients to the adverse events associated with an intravitreal injection (Chapter 1.14). Therefore, monthly treatment may be impractical in routine clinical practice and alternative strategies were investigated. Subsequent treatment trials aimed to reduce this burden of treatment by reducing the frequency of injections.
1.12.3.1.2 - An effective treatment regime with less frequent injections

The Phase IIIb, Multicentre, Randomised, Double- Masked, Sham Injection-Controlled Study of the Efficacy and Safety of Ranibizumab (PIER) (125, 126) assessed the efficacy of 0.3mg and 0.5mg dosing on patients with nAMD with classic or non-classic angiographic appearance. It was one of the first trials attempting to establish an effective regime with a fixed treatment interval greater than 1 month. Patients were injected with a monthly loading dose over 3 months, followed by reinjections every 3 months. These patients were found to have an improvement compared to sham injections, however the results were inferior to the MARINA and ANCHOR trials. The Efficacy and Safety of Ranibizumab in Patients With Subfoveal CNV Secondary to ARMD (EXCITE) study also assess the role of quarterly vs. monthly injections, with results consistent with the PIER study findings. (127)

Secondly, in the PIER study VA was found to improve once patients were switched from quarterly dosing to monthly dosing, (126) indicating that more frequent administration was linked to better outcomes. The HORIZON study found a similar outcome from the opposite perspective, in that switching patients from a monthly schedule to a less frequent, investigator determined as-needed dosing (pro re nata, (PRN)) resulted in poorer visual outcomes. (128)

The PIER study also established that there was no difference in the proportion of patients who improved their VA >15 letters when administered 0.3 vs. 0.5mg of ranibizumab. This finding helped established the accepted dose of 0.3mg via intravitreal injection. Furthermore, PIER found that patients who were switched from the sham injection arm for 1 year to the treatment arm continued to experience a decline in their vision. This suggests the importance for early treatment of nAMD lesions would result in superior outcomes.
1.12.3.1.3 - Incorporating clinical indicators for re-treatment

The Prospective OCT imaging of Patients with Neovascular AMD Treated with Intra-Ocular Lucentis (PrONTO) study aimed to reduce the burden of injections by using a clinical algorithm to guide retreatment on a PRN basis with monthly visits irrespective of the clinical findings in the previous month. (129) This algorithm included VA, OCT, fundus, and FFA features. The criteria for retreatment was as follows:

Any one of the following when compared with the previous month’s data:

1. At least 5 letters VA loss with OCT evidence of fluid at the macula
2. Increase in OCT central thickness of at least 100um
3. New macular haemorrhage (clinical exam or photographic)
4. New area of classic choroidal neovascularization on FFA
5. Persistent fluid on OCT 1 month following a previous injection.

The PrONTO study protocol mandated the established loading dose of 3 monthly injections prior to application of its clinical algorithm for retreatment. The 24-month results showed average VA gains of 11.1 letters comparable with the MARINA and ANCHOR trials, with a much-reduced number of injections of 9.9 injections over 24 months. This compared favourably with the MARINA and ANCHOR trials, which required 24 injections over 24 months. (129) Although the sample size was small (n=40) it helped shift the paradigm surrounding injection frequency, indicating that not all patients required monthly injection to achieve an optimal outcome, and that the use of additional clinical indicators was helpful. Whilst the injection frequency was reduced, (and therefore the risks and costs associated with injection also reduced), burdensome monthly visits were still required in this protocol.
The criteria for retreatment incorporating OCT features in the PrONTO study was an evolutionary step towards further PRN protocols used in subsequent studies, in addition to the current most practiced treatment regime in Australia, treat and extend (TaE).

In the large, multicenter CATT (Comparison of Age-Related Macular Degeneration Treatments Trials), the efficacies of ranibizumab and bevacizumab were compared to each other, and different treatment regimes within each drug were compared (OCT-guided PRN dosing vs. fixed monthly retreatment strategies over 2 years). Although fixed monthly retreatment led to greater mean visual acuity gain, the VA differences between PRN (+8.8 letters) and monthly (+6.7 letters) groups were considered as not significantly different for the ranibizumab groups (according to the non-inferiorly margin predefined as 5 letters). (118) When PRN patients of both drugs were compared to monthly treated patients of both drugs, there was a 2.4 letter superior outcome in mean VA with ranibizumab. Bevacizumab and Ranibizumab were found to have equivalent visual outcomes at 2 years when used with the same dosing regime, with the visual gains within 1.4 letters of each other between drugs. The greatest difference in mean VA gain was between ranibizumab monthly and bevacizumab PRN (3.8 letters).

The CATT suggested inferior anatomical outcomes with bevacizumab, which had an OCT fluid free status of 13.9% and 22.3% for PRN and monthly regimes vs. 30.2% and 45.5% for the corresponding ranibizumab groups. The resolution of fluid as a treatment outcome, and its effect on visual function is the subject of recent clinical trials. (130)

The Phase III, double-masked, multicenter, randomised, active treatment-controlled study of the efficacy and safety of 0.5 mg and 2.0 mg Ranibizumab administered monthly or on a PRN basis in patients with subfoveal neovascular age-related
macular degeneration (HARBOR) comparing monthly ranibizumab to a PRN regime over 2 years. (131) Mean visual acuity was +9.1 letters compared to baseline in the monthly 0.5mg group compared to +7.9 letters in the 0.5mg PRN group. Of note, the number of injections in the PRN arm was 13.3 over 2 years, with 5.6 injections in year 2. The PRN group did not meet the pre-determined non-inferiorly margin of 4 letters, therefore PRN dosing was not considered to be equivalent to monthly dosing, although a meaningful clinical improvement was achieved in both groups. This is despite similar mean 1-year VA results to the equivalent arm of the CATT at 1 year, which had a different non-inferiorly margin of 5-letters, and concluded that PRN dosing was non-inferior to a monthly regime. (132)

Within the HARBOR study, there was a large variation in the number of injections required in their PRN treatment group was reported (3 – 24 injections over 2 years). This suggests that some patients will be under or over treated if a fixed treatment regime was adopted. There are few baseline markers that predict the frequency of injections required over 5-years of treatment.
1.12.3.1.4 - Treat and extend regime

Treat and extend involves fixed treatment intervals until clinical remission, (as determined by OCT and other clinical findings similar to the PrONTO protocol) followed by an increasing treatment/review interval. There are several variations of this treatment protocol, with one common regime involving reviews and injections progressively extending by 2 weekly intervals if there are no signs of disease activity, until a maximum of 3 months. If there are markers of disease activity, the treatment interval is reduced by 2 weeks.

Scarce data is available about the outcomes of this treatment regime. The first prospective study of a 12-month TaE regime was published in 2013 by Toalster et.al. (133) They reported a mean VA improving from Snellen 20/62 at baseline to 20/46 at 12-months with a mean of 8 injections per patient. Abedi et al. (134) conducted a 2-year prospective study, and reported an improvement of +9.5 letters at 12-months (Mean injections 8.6) and 8.0 letters at 24-months (mean injections 5.6 in year 2). In both of these studies, a dry macula was required before extension (defined as an absence of fluid on OCT or haemorrhage). Both prospective TaE studies provided encouraging data to suggest that such a regime can produce clinically meaningful outcomes, whilst markedly reducing the injection burden. Prospective studies comparing an TaE protocol with a PRN protocol are lacking, but would further clarify the best flexible treatment regime. If TaE is found to be non-inferior to PRN, resulting in a shift of all patients to TaE, it would markedly reduce the number of visits required for patients, whilst maintaining the flexibility of treating patients according to the large variation in numbers of injections required.
1.12.4 - Aflibercept

Aflibercept (Eylea, Regeneron, Tarrytown, NY) differs from other anti-VEGF agents in its mechanism of action, as it is a VEGF receptor fusion molecule that binds to the VEGF ligand (with a higher affinity than the native VEGF receptor). Its binding affinity is higher than ranibizumab and bevacizumab. (135) It also binds PGF, which is present on endothelial cells and leucocytes. (135) Its arrival initially brought hope for a drug that would decrease the injection burden for many patients compared to the established agents at the time (ranibizumab and bevacizumab).

The phase 3 VEGF trap-eye: Investigation of efficacy and safety in wet AMD study (VIEW)-1 and VIEW-2 studies (136) were the landmark trials assessing efficacy of aflibercept vs. ranibizumab. The treatment arms were aflibercept 0.5mg monthly, aflibercept 2.0mg monthly, aflibercept 2.0mg every 2-months following 3 initial monthly doses, and 0.5mg ranibizumab monthly. Findings from baseline to 52 weeks were that 2-monthly 2mg aflibercept was not-inferior to monthly 0.5mg ranibizumab (control arm). The non-inferiority criterion applied to the study was the proportion of patients maintaining their visual acuity having less than 10% variance.

Between 52 and 96 weeks, the treatment interval for all 4 treatment arms was changed to PRN dosing. For weeks 52 – 96, an average of 4.2 injections were required for 2.0mg aflibercept and 4.7 injections for 0.5mg ranibizumab during the second year of the study. Visual acuity results were considered to be equivalent between these 2 arms, although there was a slight reduction in the mean VA of all arms. It is noteworthy that the injection interval during the second year of the study was capped at 12 weeks irrespective of disease inactivity, suggesting that the treatment interval could have been extended further for both ranibizumab and aflibercept. The driver behind the higher number of average injections in ranibizumab was a higher proportion of these patients that required intensive treatment,
suggesting that those with greater disease activity may require fewer injections with aflibercept. (137)

Aflibercept has been shown to have a role in nAMD patients resistant to treatment with regular ranibizumab therapy. Chang et.al published a prospective study with 49 treatment resistant patients switched to aflibercept. They found a 4.7 letter improvement along with a 97.2um reduction of central retinal thickness at 12-months compared to baseline. (138)
1.12.5 – Continuing challenges of anti-VEGF therapy

Despite the significant progress made in the age of anti-VEGF agents, it is not a panacea for nAMD. A sizeable proportion of patients are non-responders, whilst others suffer a recurrence should treatment cease, often in an unpredictable fashion. (139) Regular visits to an eye clinic are mandated, and treatment may be lifelong which results in an extremely burdensome exercise for the patient and their families. There are still a lack of reliable baseline biomarkers for nAMD recurrence, and long-term prognosis, which would allow more individualised therapy (140) and aid in the counselling of patients. A treatment modality to prevent CNV formation has not been developed to date, which would represent the ultimate remedy for nAMD’s potentially blinding outcomes.
1.13 - Safety concerns of anti-vascular endothelial growth factor agents and its administration

There are safety concerns related to the ocular and systemic consequences of inhibiting VEGF by agents such as ranibizumab. Secondly, there are concerns surrounding adverse events related to administration of intravitreal injections.

1.13.1 – Anti-vascular endothelial growth factor crossing into the systemic circulation, and the differences between ranibizumab and bevacizumab:

Bakri et.al (117) demonstrated in an animal model that 1.6% of the injected bevacizumab dose crossed into the systemic circulation. In 2009, Rosenfeld at Bascom Palmer Eye Institute reported a treatment effect on nAMD from systemically administered bevacizumab. This demonstrated that bevacizumab could cross from the systemic circulation to the ocular circulation, validating concerns that systemic absorption of the drug may lead to drug-mediated systemic adverse events. (141) This issue is also thought to be relevant for ranibizumab, although caution should be exercised in extrapolating safety data of one anti-VEGF agent to another due to biochemical differences in the agents and their effects at the molecular level. (142)

Accepting that these intra-vitreal agents can cross into the systemic circulation, and that bevacizumab has a longer half-life than ranibizumab(143), it remains unclear whether this intra-ocular concentration is sufficient to mediate a systemic adverse event, and whether there is a difference between these agents. Several recent randomised control head to head trials between bevacizumab and ranibizumab have demonstrated a statistically significant increase in adverse events over baseline with either agent, without a difference between drugs. (118, 119) The lack of difference between drugs is also supported by long-term surveillance studies of patients continuing treatment after clinical trials such as the HORIZON study (an open label extension trial of patients treated with ranibizumab in the ANCHOR, MARINA and
FOCUS studies), which showed no increased rate of adverse events compared with baseline rates. (128) The CATT study did however note an increased risk of serious adverse events with bevacizumab (relative risk (RR) 1.29 compared to ranibizumab). It is critical to note that the CATT was not statistically powered to assess differences in safety. Since the CATT was completed, there have been several other randomised controlled trials comparing ranibizumab with bevacizumab. A Cochrane review did not find a difference in serious systemic adverse events between these anti-VEGF agents. (144)

1.13.2 - Systemic adverse events related to ranibizumab

All anti-VEGF therapies carry a theoretical risk for systemic thromboembolic events. The antiplatelet Trialists’ Collaboration defines these to include haemorrhagic and ischemic cerebrovascular conditions, myocardial infarction. In HORIZON and SECURE studies (both open label extension trials of ranibizumab), the proportion of patients with these systemic events was 5.6% and 5.3% over 4 years.

Vascular endothelial growth factor has a physiological role in vascular homeostasis via mediation of nitric oxide causing vasodilation of vessels, and by promoting neovascularisation. Vascular endothelial growth factor blockade can therefore increase blood pressure. (145) In the HORIZON and SECURE studies, hypertension affected 8.7% and 9.0% of the study populations respectively. (128) (146)
1.13.3 - Ocular adverse events

The SAILOR study (147) is one of the largest Phase III studies to assess the incidence of adverse events (AE) with ranibizumab therapy over 12-months, with a total cohort of 4300 patients. With regards to serious adverse events, rates quoted were traumatic cataract (0.08%), retinal detachment (0.1%), retinal tear (0.1%), endophthalmitis (0.2% - 0.4%, both cases were presumed with no organism isolated) vitreous haemorrhage (0.9%), and ocular inflammation (1.0% - 1.5%, all figures expressed as a % of all patients). Interestingly the study did not report the rate of IOP elevation, a recognised adverse event from intravitreal injections.

The LUMINOUS study was post-marketing surveillance research that collected safety data from patients treated with ranibizumab in the real-world clinical setting. This study included 4444 patients, with the serious ocular events included RPE tear (0.61% of patients), intra-ocular pressure events (0.27%) and traumatic cataract (0.23%). (148)
1.13.4 - Measures to decrease the incidence of endophthalmitis

The use of antibiotics pre-injection, immediately after injection and in the days after injection has not been shown to reduce the risk of infection. (149) Some studies have shown that the routine use of prophylactic antibiotics can promote antibiotic resistance and increase the risk of endophthalmitis. (150)

An expert panel recommends povidine-iodine use. There are no controlled trials comparing endophthalmitis rates between patients with and without povidine iodine, however the evidence base cited draws on data from cataract surgery with and without povidine iodine. In these cataract studies, the use of povidine – iodine was associated with a reduction of infection risk. (149)

Eye speculums or manual retraction of the eyelids are recommended for intravitreal injections. There is no evidence that a speculum is superior to manual retraction. (151) The main goal is to prevent contact of the eyelash/eyelid margin with the injection needle to prevent the intraocular introduction of commensal organisms

The use of facemasks for the injecting physician may reduce the risk of endophthalmitis. An in vitro study by Wen et.al (152) investigated the rate of bacteria grown on culture media when subjected to a volunteer speaking with and without a face mask for 5 minutes, and standing in silence for 5 minutes. Significantly greater amounts of streptococcal species were grown in the agar plates exposed to 5 minutes of talking without a facemask. (152) Turning the face whilst speaking did not significantly reduce the risk of this growth, and therefore the recommendation from this study is for injecting personnel to wear a face mask, and to ask the patient to minimise talking during the procedure.
1.14 - Long – term outcomes of ranibizumab therapy

At the time of the LTRS design, there was limited long-term data on outcomes following ranibizumab treatment for AMD, in a non-trial clinical setting, with disease activity guided retreatment. The HORIZON extension study published by Singer in 2012 reported 60 month adverse events outcomes from 73 patients who were formerly enrolled in the ranibizumab treatment arms, and 43 patients previously enrolled in the control arms of three randomised controlled trails, (MARINA, ANCHOR, FOCUS.) (128) Given that these patients started treatment whilst on a clinical trial, with fixed, monthly treatment protocols for the first 24 months, the HORIZON extension results outcomes would not reflect the majority of real world clinical practice, where both frequency of injections and number of follow-up visits were far less than 12 per year in the first 2 years following diagnosis.

Clinical studies that had reported long-term (4-year) visual outcomes had small numbers based on a “survival analysis” method of analysis. In 2013, Fauk et.al. reported 7 patients with data for 48 months (153), and in 2012 Pushpoth et.al. reported results from 110 patients with 48 months of follow-up, of which 97 had 48-month vision data (approximately 10% of the original treated cohort). (154) Pushpoth found stable vision in 82% of patients at 4-years (defined as not losing >15 letters compared to baseline).
1.15 - Other issues surrounding management of neovascular age-related macular degeneration with anti-vascular endothelial growth factor agents

1.15.1 - Does anti-vascular endothelial growth factor treatment in the first eye result in a benefit to the second eye?

There is a notion that anti-VEGF agents administered by the intra-vitreal route of one eye can cross into the second eye via the systemic circulation, and therefore exert a prophylactic or therapeutic effect on diseases that are mediated by this molecule, including proliferative diabetic retinopathy and nAMD. There are several reports that support this hypothesis. (117, 155-157)

Avery reported 2 cases of proliferative diabetic retinopathy (PDR) that following injection with 1.25mg of intravitreal bevacizumab showed a reduction of fluorescein leakage in the second eye. This suggests that the second eye may receive some benefit from the anti-VEGF agent from the injected eye. (155) These findings also support the concerns surrounding the systemic toxicity and consequent cardiovascular adverse events following an intravitreal injection of anti-VEGF agent.
Bakri in 2007 studied the vitreous half-life of 0.5mg ranibizumab and 1.25mg of bevacizumab administered by intravitreal injection into a rabbit model. (117) The half-life was 2.88 days for ranibizumab vs. 4.32 days for bevacizumab. In this study, ranibizumab was not detected in the serum or second eye, whereas bevacizumab was detected in serum and the second eye, peaking in concentration between the 5th and 10th day following injection. In contrast, other clinical reports have suggested the lack of a second eye effect including Velez-Montoya et.al (141) who found no observable clinical effect in the second eyes of patient injected with bevacizumab for diabetic macular oedema (DMO) The authors did acknowledge that a limitation of their study was the small sample size. A retrospective analysis of ranibizumab in MARINA and ANCHOR trials did not show a statistically significant difference in the incidence of second eye nAMD between the treatment arms and the control arms, suggesting that there was minimal protective effect exerted on the second eye, (PDT and placebo respectively) (42) It therefore remains unclear whether second eyes received a clinically meaningful benefit from anti-VEGF agent
1.15.2 - The rate of recurrence of inactive disease

There is limited data on the rate of recurrence of a ranibizumab treated, inactive CNV lesion. Rasmussen reported that of 120 eyes that had ceased treatment over 4 years as a result of clinically inactive disease, 25 (20.1%) were subsequently referred for further treatment. (158) Vuze et.al reported a recurrence rate of 91% for nAMD in patients who had ceased intravitreal bevacizumab or ranibizumab for more than 3 months. In this cohort of 103 patients, the mean follow up period was 33.1 weeks, and no correlation was found with the size or angiographic type of the initial lesion. (159) Currently it is unknown how frequently a patient should be re-examined after apparent disease inactivity.
1.16 - Anti vascular endothelial growth factor agents - economic considerations

1.16.1 - Direct costs to the health system

As of August 2007, ranibizumab has been funded by the Australian government through the PBS, at a cost of $1976 Australian dollars (AUD) per injection, lowering to $AUD1431 on 1 April 2013. (Drug utilisation sub-committee - ARMD utilisation analysis, June 2015) By 2010, ranibizumab was the second highest costing drug on the Australian Pharmaceutical Benefits Scheme at $237 million. Totalling $AUD 173.7 million in the 2014 financial year in drug costs alone, (160) ranibizumab is currently the eighth most costly drug on the Australian PBS and combined with aflibercept ($AUD 181.6 million in 2014) forms the second most expensive class of medication on the PBS after the lipid modifying medications atorvastatin and rosuvastatin. (160) In 2014, 249,722 approved anti-VEGF injections were administered in Australia for nAMD in 2014 and this figure has been increasing since 2007. (160) There are significant additional costs to the health care system related to the administration of these injections, and the clinic based investigations and reviews necessary to guide ongoing treatment.
1.16.2 - Neovascular age-related macular degeneration related legal blindness

The nAMD phenotype of AMD is a disease with significant social burden. Two-thirds of all cases of late AMD are neovascular, and visual loss often occurs over months, in contrast to the other subtype of late AMD (geographic atrophy) where loss occurs more gradually over decades. A recent analysis of the effect of ranibizumab treatment in Australia found that over a 2-year period, treatment reduced the rate of legal blindness (Snellen VA >6/60) by 68%, (1622 cases) and visual impairment (Snellen VA <6/12) by 28% (1774 cases). (6) These results were consistent with findings of a real-world database from Denmark, which found a relative risk reduction of 50% for nAMD related blindness over the 5-years following the introduction of nAMD, compared with the preceding 5-years. (161) Although the Australian results were based on statistical modelling, and did not incorporate an economic cost/benefit analysis for Australia, it is compelling data that suggests a net economic gain for society, in addition to a functional benefit for the patient. One American analysis estimated the cost/benefit ratio for ranibizumab at $63300 United States Dollars (USD) per quality adjusted life year (QALY) for monthly dosing and $USD18600 for PRN dosing. (162) This was contrasted with the cost of bevacizumab at $USD2676/QALY for monthly dosing and $USD3333/QALY for PRN dosing.
1.16 3 - Worldwide access to ranibizumab

As of May 2012, ranibizumab (Lucentis) was fully funded by governments of a limited number of countries (US, Australia, UK, France, Germany, Japan, Brazil, Switzerland, Austria, Sweden (Novartis Pharmaceuticals, Australia, May 2012). At approximately AUD$2000 drug cost per injection in 2012, there was a wide cost gap between this medication and its alternative bevacizumab (Avastin, approximate cost $50 per injection) used in many other countries. This highlights that many countries are unable, or unwilling to fund the cost of ranibizumab whilst an alternative is available.
1.17 - Unresolved questions in neovascular age-related macular
degeneration management and thesis hypothesis

There are few real world studies on the long-term treatment of nAMD. Whilst it is
clear that ranibizumab is an effective treatment under trial conditions, its efficacy
when administered under real-world conditions is unclear. There is a movement
towards administering less injections, however the evidence suggests that this
results in inferior outcomes compared to monthly injections.\(^{(128)}\) The proportion of
patients that still require regular treatments over 5-years in the real world is also
unknown. It is assumed that there would be value for continuing monitoring and
treatment during the 5-year period. This study hypothesised that ranibizumab would
have long-term benefit in maintaining the VA and anatomical structure in patients
with nAMD using an individualised treatment regime whilst reducing the burden of
injections.

The long-term safety of ranibizumab injections is unclear, with some studies
suggesting a high endophthalmitis rate. This study hypothesised that safety profiles
recorded from long-term ranibizumab treatment in real-world setting may be more
representative of routine clinical practice compared to the short-term clinical trials.

The natural history of nAMD without treatment leads to rapid visual decline, and
intuitively it would seem that early treatment would result in superior outcomes,
supported by studies that showed good baseline visual acuity is a positive baseline
prognostic sign for final visual outcomes.\(^{(140)}\) The rate of second eye involvement is
high, and with the advent of anti-VEGF therapy and regular treatment regimes, there
is now an opportunity to regularly examine the second eye. It is unclear how often
patients are symptomatic, or are shown to have a decrease in VA, whilst there is a
sub-foveal CNVM visible on FFA. It is also unclear whether treating these early
lesions (that may be asymptomatic and have minimal impact on VA) would be beneficial. The second eye ranibizumab study (SERS) hypothesised that closely monitoring second eyes of the patients whose first eye is undergoing ranibizumab treatment will be effective in early detection and treatment of nAMD in the second eye. This is thought that second eyes would show better VA and anatomic structure findings at 1-year compared to the first eye.
1.18 - Thesis aims

The first aim is to evaluate the long-term visual and anatomical of intravitreal ranibizumab (an anti-VEGF agent) in treating nAMD by examining a cohort over 5-years within a real-world clinic setting. Furthermore, the long-term pattern and safety of ranibizumab injections for treating nAMD will be assessed.

The second aim is to compare the clinical presentation and treatment outcomes between the first and second treated eyes in patients that developed nAMD in both eyes whilst under regular clinic follow-up.
CHAPTER TWO – General methods for studies

2.1 - Introduction

This chapter outlines the methodology used to establish the overall ranibizumab retrospective database, clinic follow-up protocol and assessment of patient outcomes relevant to all arms of the thesis. Methodologies specific to the individual studies will be included in the relevant chapters.
2.2 - Patient recruitment

Sydney Retina Clinic (SRC) is a tertiary ophthalmic referral centre, primarily treating vitreo-macular disease. Patient assessment, investigations and treatment are carried out onsite in combined clinic rooms and day procedure unit.

Patients are referred by public hospital ophthalmology clinics, ophthalmologists in private practice, optometrists and general practitioners.

This study adhered to the tenets of the Declaration of Helsinki, and ethics approval was obtained from Human Research Ethics Committee, The University of Sydney. Informed consent was obtained from all participants before they were recruited into the study.

2.2.1 - Study design and establishment of the database:

The ranibizumab retrospective database was designed with two major aims:

1) To investigate the long-term (5-year) outcomes of nAMD treated with ranibizumab in a clinical setting (LTRS).

2) To investigate the clinical presentation and outcomes of patients subsequently developing nAMD in the second eye whilst undergoing regular ranibizumab treatment for nAMD in the first eye (SERS).

The study design for both LTRS and SERS was a retrospective, consecutive case series.

2.2.2 - Eligibility for subsidised ranibizumab

For eligibility for inclusion in the ranibizumab retrospective database, the patient needed to satisfy the criteria for government subsidy of nAMD treatment. In Australia, the PBS subsidises the use of ranibizumab for macular diseases. The criteria for
eligibility for subsidised ranibizumab treatment for nAMD (and therefore inclusion into the ranibizumab retrospective database) is as follows:

1 - Age > 50 years old

2 - sub-foveal CNV secondary to AMD diagnosed by FFA, with the lesion demonstrated on a retinal FFA

Patients would also qualify for PDT (a therapy now considered second-line) if their pre-treatment VA is ≤6/60, and if their CNV appearance was predominantly (>50%) classic on FFA. All patients in this study received PBS subsidised ranibizumab, as this is available irrespective of the pre-treatment VA.

2.2.3 – Inclusion and exclusion criteria

Consecutive patients who commenced treatment with ranibizumab for CNV secondary to AMD between June 2007 - May 2008 were assessed for inclusion to the study. These patients underwent treatment by a single retinal specialist (AC) at a single practice (SRC). All ranibizumab vials were supplied by one pharmacy (Oze Pharmacy) who provided a complete record of every patient who had commenced treatment on ranibizumab and this formed the basis of the ranibizumab retrospective database.

Inclusion criteria was nAMD diagnosed by fundus fluorescein angiography (FFA), commencement of intravitreal ranibizumab for nAMD between June 2007 and May 2008 and completion of 5-years of follow-up from initial ranibizumab injection, with a single physician (AC) with no follow-up interval exceeding 6 months. June 2007 coincided with the availability of ranibizumab though the Australian PBS.

Each patient record satisfying the inclusion criteria between June 2007 - May 2008 was accessed using practice management software VIP, (Houston Medical) and the
visits associated with a ranibizumab injection were identified. The baseline (first) and last date of ranibizumab injections was identified, and the time interval calculated. If this time interval was ≥5-years (60 months) for one eye (LTRS), or >12-months in each eye for those who had ranibizumab in both eyes (SERS) the patient satisfied the major inclusion criteria for the study database and a physical chart review was triggered for that patient.

For patients with two eyes meeting the inclusion criteria for the LTRS, one eye was randomly selected using a computer randomiser to be included in the study.

Patients were excluded from either the LTRS or SERS if the study eye underwent vitrectomy surgery at any time due to the increased clearance of intravitreal ranibizumab reported in animal models, (163) and reported clinically decreased efficacy of intravitreal bevacizumab in the treatment of diabetic macular oedema. (164). Additional exclusion criteria included treatment with PDT, intravitreal bevacizumab or triamcinolone during the follow-up period; or intravitreal ranibizumab prior to June 2007, and cataract surgery during the follow-up period.

2.2.4 - Extension of the ranibizumab retrospective database for the second eye ranibizumab study (SERS)

During preliminary analysis of the results for the LTRS, it was decided to expand the scope of the database to assess patients who developed nAMD in the second eye whilst being treated for nAMD in the first eye. All patients who commenced unilateral treatment with ranibizumab for nAMD at SRC between July 2007 and March 2012 were assessed for inclusion, utilising dispensing data provided by OZE pharmacy. Patients who subsequently commenced ranibizumab treatment in their second eye, with at least 12-months of follow-up for both eyes at the end of the study duration, and treated by a single ophthalmologist (AC) were included in the study. Exclusion criteria were applied as outlined in the previous section for the LTRS.
2.3 - Clinical protocols for the treatment of neovascular age-related macular degeneration

2.3.1 - Baseline clinical assessment:

“Baseline” is defined as the date of the nAMD diagnosis for patients recruited into the study. At baseline, Snellen VA, intraocular pressure (IOP) measurement and fundoscopy were conducted. Central macular thickness as measured with Stratus TD-OCT (Software Version 5.0; Carl Zeiss Meditec, Dublin CA). The presence, location and type of CNV were determined by FFA using the Spectralis (Heidelberg Industries, Germany) or Visucam non-mydriatic fluorescein camera (Zeiss Engineering, Germany). Patient medical history, concomitant medication and previous treatment for nAMD were recorded. Polypoidal choroidal vasculopathy was not screened as ICGA was not performed routinely in all cases. Indocyanine green angiography was only performed when the clinical presentation and demographics of the patient suggested PCV. Patients proven to have PCV were not included in these studies.

2.3.2 - Measurement of visual acuity at baseline and all subsequent visits

Visual acuity was measured using a standardised protocol by trained clinic staff, using Snellen VA charts projected onto a standardised background at 3 meters, with results expressed in notation to represent the common testing distance of 6 meters. When one eye was tested another eye was occluded. Visual acuity was measured using distance spectacles if available, and if a patient did not obtain 6/6 acuity, a pinhole occluder with a 1.25mm aperture was used to minimise the effect of refractive error. Baseline visual acuity was noted in Snellen VA notation, and the best vision recorded was written in the notes (either without correction, with spectacle correction, or pinhole correction). If the acuity was worse than 6/60, a stepwise
measurement of 3/60, 2/60 and 1/60 was used with a mobile Snellen VA card. If necessary, counting fingers, then hand motions and perception of light was assessed at 30cm.

Visual acuity was measured at every visit. For the purposes of analysis, and to aid comparison of results with established clinical trials which used early treatment of diabetic retinopathy study (ETDRS) VA charts, Snellen visual acuity was converted to a decimalised value, then to “approximate ETDRS letters” using the following formula described by Gregori and Rosenfeld. (165)

\[ \text{Approximate ETDRS Letters} = 85 - 50 \times \log (\text{Decimalised Snellen VA}) \]

A conversion table for Snellen VA to approximate ETDRS letters is included as Table 3.
Table 3 - Conversion table of Snellen visual acuity to Approximate ETDRS Letters

<table>
<thead>
<tr>
<th>Snellen VA</th>
<th>Approximate ETDRS Letters</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/6</td>
<td>85.00</td>
</tr>
<tr>
<td>6/7.5</td>
<td>80.15</td>
</tr>
<tr>
<td>6/9</td>
<td>76.20</td>
</tr>
<tr>
<td>6/12</td>
<td>69.95</td>
</tr>
<tr>
<td>6/15</td>
<td>61.14</td>
</tr>
<tr>
<td>6/18</td>
<td>54.90</td>
</tr>
<tr>
<td>6/21</td>
<td>50.05</td>
</tr>
<tr>
<td>6/24</td>
<td>54.90</td>
</tr>
<tr>
<td>6/30</td>
<td>50.05</td>
</tr>
<tr>
<td>6/36</td>
<td>46.09</td>
</tr>
<tr>
<td>6/48</td>
<td>39.84</td>
</tr>
<tr>
<td>6/60</td>
<td>35.00</td>
</tr>
</tbody>
</table>
2.3.3 – Optical coherence tomography image acquisition for baseline and subsequent follow-up visits

From June 2007 until March 2009, OCT was performed using a Stratus TD-OCT with trained clinic staff using the Fast Macular Thickness Map protocol, capturing 768 A-scans in a 6mm “star” arrangement (128 A-scans radially arranged 60 degrees apart.(166) Due to advancements in OCT imaging technology, SRC upgraded their device to a Cirrus SD-OCT system (Software version 4.0, Carl Zeiss Meditec, Dublin CA) (Figure 22). The “macular cube protocol” capturing 65536 A-scans in a 6mm cube (512x128 A-scans in a continuous cube arrangement was used for all scans. Additionally, each patient scanned on the Cirrus system also underwent a “5-line high-definition Raster protocol”, which captured 5 high resolution scans aimed in the foveal region. Patients in the LTRS cohort transitioned to the Cirrus SD-OCT system between March and May in 2009, corresponding to a time point within the 9 – 24 month window of follow-up from baseline. OCT images since 2011 were performed on either the Cirrus or Spectralis OCT scanner. (Figures 22-23)
Figure 22 - Cirrus spectral-domain optical coherence tomography scanner at Sydney Retinal Clinic
Figure 23 - Spectralis Heidelberg retinal angiograph (HRA) (Heidelberg Engineering) used for FFA studies at Sydney Retina Clinic
Figure 24 - Fluorescein dye used for angiography
2.3.4 - Clinical assessments at follow-up visits

After a 3-month loading period consisting of monthly visits and injections, patient follow-up intervals varied between 1 to 6 months depending on disease activity. At each visit, Snellen VA measurement, OCT and ophthalmic examination of both eyes were performed. FFA and IOP measurement were performed at the discretion of the treating physician. The most common indication for repeat FFA was persisting fluid seen on OCT refractory to monthly treatment. Repeat IOP measurement was performed as a yearly screen, or when the clinical assessment showed evidence of IOP increase after the treatment. ICG was performed if there was a clinical suspicion of idiopathic PCV.

Systemic and ocular AE and SAE were documented from direct patient interview and recorded in the clinic notes by clinic staff at each clinic visit. Ocular AE were confirmed by eye examinations at the clinic visit. Systemic AEs were reported by patients and confirmed by the patient’s general practitioner or other treating physician.
2.3.5 - Ranibizumab injection protocol and follow-up visits

Injections were performed under strict asepsis and standardised protocol by a single clinician (AC). (167) Patients were prepared via administration of topical anaesthesia (Benoxinate hydrochloride 0.4%), followed by application of a topical antimicrobial solution (Povidine iodine 5% [Sanofi, Paris, France] or chlorhexidine 0.1% [Pfizer, New York, NY] in the case of documented prior iodine allergy) to the conjunctival sac. The injection of 0.5mg of ranibizumab (in 0.05ml) was administered using a 30-gauge needle through the pars plana. Patients were prescribed topical chloramphenicol solution 0.5% 4 times daily for 1 week after injection. A follow-up phone call to monitor AEs was conducted for all patients within 24 hours of injection.

All patients received three initial monthly loading doses of 0.5mg ranibizumab and subsequent treatment was at the physician’s discretion, with a minimum of 28 days between injections.

Criteria for re-treatment included one or more of the following: reduction in Snellen VA of ≥1 line; persisting exudation or blood at the macula on clinical examination; presence of SRF or IRF on OCT; or development of new areas of CNV on FFA.

Patient follow-up was initially monthly, then less frequently if clinically deemed safe to do so based on these criteria.
2.4 - Source data collection

Data collection sheets for baseline patient characteristics were developed and refined after several trial runs of data collection. The finalised data collection sheets for the LTRS and SERS are attached as Appendices A - E. Once a patient had been identified for inclusion into either study, their physical medical record was reviewed, and data was entered onto the relevant data sheet.

2.4.1 - Baseline visit for long term ranibizumab study and second eye ranibizumab study

The first ranibizumab injection date for first and second treatment eyes was identified via the practice management electronic database. The review of the physical patient file identified the clinic visit associated with the decision to initiate ranibizumab therapy, and this was deemed the “baseline visit.” The clinic record was reviewed for the presenting visual symptoms, VA, previous treatment for AMD and other macular conditions, notated on the baseline data collection sheet (Appendix A). Potential baseline predictors of response to ranibizumab were identified, including previous ocular treatments for nAMD (focal laser, PDT, bevacizumab) concurrent medical conditions that may have confounded the results of systemic adverse events (ischemic heart disease, stroke, hypertension, hypercholesterolemia, diabetes).

Quantitative (CMT) and qualitative OCT data was collected by a review of the relevant baseline OCT archived images. This is detailed in chapter 2.5.
2.4.2 - Follow-up data points (including optical coherence tomography)

For the LTRS, additional data points were 1,2,3,6,12,18,24,30,36,42,48,54 and 60 months following baseline treatment with ranibizumab. Best corrected VA was collected from the patient files, along with the model of OCT machine used. (Appendix B). Optical coherence tomography qualitative and CMT data was collected from the relevant archived scans at the 60 months following baseline injection.

For the SERS, VA was collected for follow-up data points of 1,2,3,6 and 12-months after the baseline treatment date for the second eye. OCT data was collected from the archived scans corresponding to the 12-month visit (Appendix F). Specifics of OCT data collection are outlined in Chapter 2.4.

The patient visit closest to each of these time points was chosen for data collection and analysis. If the visit occurred >1 month before or after the data point, the visit was classified as “missing data”.

2.4.3 - Adverse events

Every visit related to an injection over 60 months for the LTRS, and 12-months for the SERS (including the data collection points defined in Chapter 2.4.2) was reviewed to record AE and SAE. The clinic protocol mandated the questioning and recording of all adverse events at every patient interaction.
2.5 – Optical coherence tomography data grading and analysis

Archived baseline OCT scans (all performed with Stratus TD-OCT for the LTRS cohort, and some performed with the Cirrus SD-OCT for the SERS cohort) and 5-year scans (97.8% from Cirrus SD-OCT and 2.2% from Spectralis SD-OCT) were evaluated by two trained image graders independently, and a third grader adjudicated disagreement. For baseline TD-OCT scans, the fast macular thickness map protocol was used, comprising of six 6mm radial lines 60 degrees part, with 128 A-scans in each line. The fovea was assessed for accurate centration within the Early Treatment Diabetic Retinopathy Study (ETDRS) grid, and the CMT was recorded from the central 1mm zone within this grid. For each of the six radial lines in each scan, inner and outer retinal segmentations defined by the Stratus automated algorithm were reviewed and redefined if necessary. If these segmentation lines did not correspond to the first inner hyper-reflective layer [representing the internal limiting membrane (ILM)] and the inner red hyper-reflective band of the larger outer hyper-reflective layer (representing the photoreceptor inner segment/outer segment, or IS/OS junction), they were manually redrawn with a computer mouse (168, 169) and validated by 2 independent observers. Central macular thickness (central 1mm zone) was then recalculated after these segmentation lines were redrawn using the Stratus version 5.0 review software. (Figures 25 and Figure 26)
Figure 25 - Stratus TD-OCT baseline scan with poor automated inner and outer retinal boundary demarcation lines
Figure 26 - Stratus TD-OCT baseline scan with inner and outer retinal boundary demarcation lines manually corrected from Figure 25. Note that the central 1.0mm macular thickness has changed from 189um (pre correction Figure 25) to 196um.
If the inner and outer retinal boundaries were unclear (e.g. significant sub-retinal fibrosis, or massive macular oedema exceeding the maximally measured retinal thickness) the scan was excluded from analysis. If the fovea was not centred, one of the 6 scans transecting the fovea was selected, and a manual calliper calculation of CMT utilising 3 points representing the central fovea and 500μm either side was recorded (Figures 27-29). If the fovea could not be identified, the scan was excluded from analysis.

The presence and the type of fluid in OCT at baseline were also recorded by individually reviewing each of the 6 scan lines from the fast macular thickness map protocol.
Figure 27 - The above image shows the foveal pit, which is decentred from the expected position of A-Scan 64. A-Scan 52 has been selected as the centre of the foveal pit, and therefore the centre of the 1mm central macular thickness. In this case, the retinal thickness is 125μm
Figure 28 - The second retinal thickness point has been selected at A-scan 42, representing the nasal border of the 1mm central macular thickness in the left eye.
Figure 30 - The third retinal thickness point has been selected at A-scan 22, representing the temporal border of the 1mm central macular thickness in the left eye. All three retinal thicknesses in Figures 27, 28 and 29 were averaged to yield a more accurate 1mm central macular thickness compared to if the automated algorithm was used.
For 5-year OCT scans, (Cirrus and Spectralis) foveal ETDRS grid alignment was adjusted if necessary, so that the 6mm grid was centred over the fovea. The CMT (computer determine average retinal thickness based on all A-scans occupying the central 1mm zone) was recorded from the aligned grid. Five-line high-definition (HD) raster scans were reviewed to qualitatively describe macular fluid type. (170)
2.6 - Fundus fluorescein angiography grading

Fluorescein angiography baseline images were reviewed and the CNV lesion classified as either occult or classic, then the total lesion size was measured using the software inbuilt callipers. As follow-up FFA was performed when required, only baseline type and location of CNV were analysed. For inclusion in these studies, CNV lesions had to show activity in the sub-foveal region (500um from the centre of the foveal avascular zone), evidenced by hyperfluorescence involving this region. (Figure 30) Two trained graders independently graded images and a third grader adjudicated disagreement.
Figure 30 - Fundus fluorescein angiography showing hyperfluorescence involving the foveal avascular zone.
2.7 - Source data entry and verification

All collected data was entered into Epidata Entry (Epidata Software, Denmark) by 2 independent data entry personnel, using a double-entry technique (each set of data entered once by 2 different data entry personnel.) Thirty-nine thousand and five hundred data points were entered from 250 patient records. Double-entry data verification (comparing each of the 2 data sets to the other) was conducted, with the Epidata software detecting discrepancies in 1417/39500 data points from 214/250 records. All of the 1417 discrepancies were checked against the data collection sheets, and patient files if necessary, and corrected. During the data collection phase, a random audit of 5% of the source data was performed and checked against the original data files.

2.8 - Statistical methods

Snellen VA was converted to ETDRS letters for statistical analyses as described above. Paired t tests were used to assess changes in VA and CMT over 5-years in the LTRS, and over 1 year in the SERS. Changes in VA compared to baseline were classified into three categories of stable (loss or gain of ≤15 letters), improved (gain of >15 letters) and worse (loss of ≥15 letters), corresponding to reports from the landmark trials. (17, 77) Two sampled t-tests were used in the SERS to assess differences in mean VA between the first and second treated eyes.
Baseline VA was stratified into five groups according to criteria of the Australian Modification of the 10th revision of the International Classification of Diseases:

1. legal blindness: baseline VA < 35 letters (Snellen VA < 6/60),
2. low vision: VA ≥ 35 (≥6/60) and < 60 letters (<6/18),
3. reduced vision: VA ≥60 (≥6/18) and < 70 letters (<6/12),
4. mildly reduced vision to Australian legal driving limit: VA ≥70 and < 85 letters (≥6/12 and <6/6) and
5. normal vision: VA ≥85 letters (≥6/6).

Changes in VA and CMT were evaluated separately in each stratified baseline VA group, to address the potential confounding effects of “ceiling and floor effect” resulting from good or poor baseline vision. One-way analysis of variance (ANOVA) was used to compare means of VA and CMT changes, respectively over the 5-years across the 5 baseline vision groups.

Linear regression was used to assess the effects of age, gender, number of injections, previous treatment, medical history, medications and baseline VA on both VA and CMT changes. The relationships between treatment outcomes and fluid type or CNV type were also analysed.

Visual acuity and CMT changes over a 5-year period on previous treatment naïve patients were evaluated, and an ANOVA was performed to compare changes in VA and CMT respectively between treatment naïve patients and non-treatment naïve patients.

All analyses were carried out using the Statistical Package for Social Sciences (SPSS, version 19), and a p-value of 0.05 was used to signify statistical significance in these studies.
CHAPTER THREE - Long-term outcomes of intravitreal ranibizumab for neovascular age-related macular degeneration in clinical practice

3.1 - Background

There is limited data available on long-term outcomes of eyes treated with an individualised treatment regime. (153, 154, 158) As patients often continue to undergo treatment with anti-VEGF agents for many years, the long-term safety and efficacy of therapy under such treatment protocols are clinically relevant, and are currently unknown. (171)

The LTRS aimed to evaluate visual and anatomical outcomes of an individualised regime of ranibizumab therapy on patients with nAMD over 5-years. The baseline prognostic factors of long-term treatment response and the safety profile of treatment were also assessed.
3.2 - Methods

3.2.1 - Study design

This is a retrospective study of consecutive patients treated with intravitreal ranibizumab for subfoveal nAMD. The study was conducted in a single tertiary referral retinal clinic and all patients were assessed and treated by a single physician (AC) according to standardised retreatment criteria.

3.2.2 - Patient eligibility

Inclusion and exclusion criteria for the LTRS are outlined in Chapter 2.2.

3.2.3 - Patient assessment at baseline visit and follow-up visits

At each visit, Snellen VA, OCT, ophthalmic examination and fundoscopy were performed. OCT findings were used as a guide for treatment as described in Chapter 3.2.5. At the 5-year visit, OCT scans were performed using either a Cirrus or Spectralis OCT. Please refer to Chapter 2.3 for further details.

3.2.5 - Treatment regime

Injections were performed in an operating theatre under a standard protocol. All patients received three initial monthly loading doses of 0.5mg ranibizumab and subsequent treatment was at the physician’s discretion.

Criteria for re-treatment included one or more of the following: reduction in Snellen vision of ≥1 line; persisting exudation or blood at the macula on clinical examination; presence of SRF or IRF on OCT; or development of new areas of CNV on FFA.
3.2.6 - Optical coherence tomography analysis

Baseline scans (all using Stratus TD- OCT) and 5-year scans (97.8% from Cirrus SD-OCT and 2.2% from Spectralis SD-OCT) were evaluated by two trained image graders independently, and a third grader adjudicated disagreement. CMT and qualitative OCT findings (IRF, SRF) were assessed and recorded. Further details on the technical aspects of the scan analysis are described in Chapter 2.5.

3.2.7 - Fundus fluorescein angiography grading

Grading was performed as described in Chapter 2.6.

3.2.8 - Statistical methods

Snellen VA was converted to ETDRS letters for statistical analyses (165). Paired t-tests were used to assess changes in VA and CMT over 5-years.

One-way analysis of variance was used to compare means of VA and CMT changes, respectively over the 5-years across the 5 baseline vision groups.

Linear regression was used to assess the effects of age, gender, number of injections, previous treatment, medical history, medications and baseline VA on both VA and CMT changes. The relationships between treatment outcomes and fluid type or CNV type were also analysed. Further details are outlined in Chapter 2.8.
3.3 - Results

3.3.1 - Overview:

Of 886 patients with nAMD who commenced ranibizumab treatment between June 2007 and May 2008, 369 completed 5-years follow-up, and 208 eyes from 208 patients were included in the study. (Figure 31) Patient general baseline characteristics are presented in Table 4, and ocular baseline characteristics in Table 5.
Figure 31 - Schematic diagram of patient selection showing inclusion and exclusion from the study analyses.
Table 4 – General baseline characteristics of patients with neovascular age-related macular degeneration that completed 5-year follow-up

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD) or Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General</strong></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>78.4 (7.2)</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>65/208 (31.3)</td>
</tr>
<tr>
<td>Study Eye (right)</td>
<td>114/208 (54.8)</td>
</tr>
<tr>
<td>Baseline Visual Acuity (letters)</td>
<td>53.6 (19.3)</td>
</tr>
<tr>
<td><strong>Medical History</strong></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular Disease</td>
<td>31/208 (14.9)</td>
</tr>
<tr>
<td>Stroke</td>
<td>19/208 (9.1)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>143/208 (68.8)</td>
</tr>
<tr>
<td>Hypercholestraemia</td>
<td>95/208 (45.7)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>25/208 (12.0)</td>
</tr>
</tbody>
</table>

SD = standard deviation
*Mean (SD)
†8/208 (3.8%) had cataract surgery in the study eye during the 5 year treatment period
‡Missing participant data
Table 5 - Ocular baseline characteristics of patients with neovascular age-related macular degeneration that completed 5-year follow-up

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD) or Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Choroidal Neovascularisation Subtype</strong></td>
<td></td>
</tr>
<tr>
<td>Occult</td>
<td>124/170 (72.9)</td>
</tr>
<tr>
<td>Minimally Classic</td>
<td>32/170 (18.8)</td>
</tr>
<tr>
<td>Predominantly Classic</td>
<td>9/170 (5.3)</td>
</tr>
<tr>
<td>Classic</td>
<td>5/170 (2.9)</td>
</tr>
<tr>
<td><strong>Optical Coherence Tomography</strong></td>
<td></td>
</tr>
<tr>
<td>Baseline Central Macular Thickness (CMT)</td>
<td>256.7 (88.0)*</td>
</tr>
<tr>
<td>Intra-retinal Fluid (IRF)</td>
<td>97/208 (46.6)</td>
</tr>
<tr>
<td>Sub-retinal Fluid (SRF)</td>
<td>9/208 (4.3)</td>
</tr>
<tr>
<td>Mixed IRF/SRF</td>
<td>65/208 (31.3)</td>
</tr>
<tr>
<td>No Fluid</td>
<td>12/208 (5.8)</td>
</tr>
<tr>
<td>Data not available</td>
<td>25/208 (12.0)</td>
</tr>
<tr>
<td><strong>Lens Status (study eye only)</strong></td>
<td></td>
</tr>
<tr>
<td>Phakic</td>
<td>145/208 (69.7)+</td>
</tr>
<tr>
<td>Pseudophakic</td>
<td>63/208 (30.3)</td>
</tr>
<tr>
<td><strong>Treatment (study eye only)</strong></td>
<td></td>
</tr>
<tr>
<td>Treatment Naive</td>
<td>71/208 (34.1)</td>
</tr>
<tr>
<td>One or more previous nAMD treatment</td>
<td>137/208 (65.9)</td>
</tr>
<tr>
<td><strong>Previous Treatment Methods (study eye only)</strong></td>
<td></td>
</tr>
<tr>
<td>Photodynamic Therapy</td>
<td>79/208 (38.0)</td>
</tr>
<tr>
<td>Focal Laser</td>
<td>29/208 (14.0)+</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>114/208 (54.8)</td>
</tr>
<tr>
<td>Bevacizumab and Photodynamic Therapy</td>
<td>54/208 (26.0)</td>
</tr>
</tbody>
</table>

SD = standard deviation
*Mean (SD)
+8/208 (3.8%) had cataract surgery in the study eye during the 5 year treatment period
‡Missing participant data
3.3.2 - Visual acuity

Mean baseline VA of the 208 eyes was 53.6 (±19.3). As shown in Figure 32, maximum visual gain occurred at 6 months with an average of 3.2 (±10.4) letter increase when compared to baseline (p<0.0005). Statistically significant visual gain of 1.9 letters (p=0.017) was maintained over 1 year. There were no significant changes from baseline VA to the end of 2, 3 and 4 years (p> 0.05 for all time points). Vision had declined slightly by 2.4 letters at 5-years (p=0.043). At the end of year 5, 23 (11.1%) patients had improved VA, 143 (68.8%) patients had stable VA, and 42 (20.2%) patients experienced VA loss (Figure 33).

Table 6 summarizes average VA changes over 5-years of the treatment in stratified baseline VA groups. Patients with poor baseline VA (<35 letters) had a greater VA improvement (+11.5 letters, p=0.01) while patients with good VA at baseline (≥ 70 and < 85) had VA that declined over 5-years (-12.9 letters, p=<0.0005).
Figure 32 - Change in visual acuity associated with ranibizumab treatment over a 5-year period.
Figure 33 – Proportion of patients with visual acuity improvement (>15 letters) VA stability (Gained <15 letters and Lost <15 letters) and VA loss (Lost >15 letters) of years 1-5 compared to baseline
Table 6 - Change in visual acuity over 5-years following the commencement of ranibizumab treatment, in relation to baseline visual acuity

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline Visual Acuity (ETDRS letters)</th>
<th>n</th>
<th>5-Year Change in Visual Acuity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td>1</td>
<td>&lt; 35</td>
<td>22</td>
<td>11.5 (5.2, 17.9)</td>
</tr>
<tr>
<td>2</td>
<td>≥ 35 and &lt; 60</td>
<td>100</td>
<td>-0.6 (-3.2, 2.0)</td>
</tr>
<tr>
<td>3</td>
<td>≥ 60 and &lt; 70</td>
<td>46</td>
<td>-3.7 (-8.2, 0.9)</td>
</tr>
<tr>
<td>4</td>
<td>≥ 70 and &lt; 85</td>
<td>34</td>
<td>-12.9 (-19.2, -6.6)</td>
</tr>
<tr>
<td>5</td>
<td>≥ 85</td>
<td>6</td>
<td>-15.8 (-51.5, 19.9)</td>
</tr>
</tbody>
</table>

ETDRS = Early Treatment Diabetic Retinopathy Study
n = sample size
CI = confidence interval
Baseline VA remained a significant predictor for VA change over 5-years, after adjusting for possible confounders of total number of injections and age (Table 7). Other baseline characteristics including age, existing medical history, concomitant medication and previous treatment history were not associated with the change in VA (data not shown).
Table 7 - Linear regression analysis of visual acuity change over 5-years.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline Visual Acuity (ETDRS letters)</th>
<th>n</th>
<th>Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Coefficient* (95% CI)</td>
</tr>
<tr>
<td>4</td>
<td>≥ 70</td>
<td>40</td>
<td>Reference</td>
</tr>
<tr>
<td>3</td>
<td>≥ 60 and &lt; 70</td>
<td>45</td>
<td>11.2 (4.9, 17.4)</td>
</tr>
<tr>
<td>2</td>
<td>≥ 35 and &lt; 60</td>
<td>100</td>
<td>16.1 (10.5, 21.6)</td>
</tr>
<tr>
<td>1</td>
<td>&lt; 35</td>
<td>23</td>
<td>30.7 (22.8, 38.6)</td>
</tr>
</tbody>
</table>

Baseline age\* | - | - | -0.1 (-0.4, 0.1) | 0.345 |

Total ranibizumab injections\* | - | - | 0.3 (0.1, 0.4) | <0.0005 |

ETDRS = Early Treatment Diabetic Retinopathy Study; n= sample size; CI = confidence interval

\* Added into regression model.

\*Change in visual acuity (number of ETDRS letters read) compared to the reference group, over a 5-year period.
3.3.3 - Previous treatment naïve patients

Fifty-eight patients (27.9%) were treatment naïve patients at the baseline. The statistical analysis indicated that there were no significant difference at 5% significance level in any VA changes or CMT change over 5-years period between patients in treatment naïve and those in non-naïve treatment group (p > 0.05).

3.3.4 - Central macular thickness

Central macular thickness data was available for 180 patients who had gradable baseline on Stratus TD-OCT and 5 year OCT measurements on Cirrus SD-OCT. A total of 28 patients had scans where the CMT could not be measured due to poor image quality. For all patients, the changeover from Stratus to Cirrus occurred between March and May 2009, which fell between the 9 and 24-month follow-up period. The transition was consistent for all patients. CMT decreased by an average of 28.3µm (P<0.0005) from baseline (256.7 ±88.0µm) to 5-years (228.4 ±77.8µm). A greater baseline CMT was associated with a greater reduction in CMT after 5-years (p<0.0005). Age, total number of injections and previous treatment was not associated with the changes in CMT, after adjusting for baseline CMT. No correlation was identified between baseline CMT and VA changes over 5-years (r = 0.082, p=0.272).

3.3.5 - Impact of baseline retinal fluid type on visual acuity and central macular thickness changes

Patients were divided into five categories with regards to their baseline fluid status. These categories were IRF (97/208 patients, 46.6%), SRF (9/208, 4.3%), mixed IRF/SRF (65/208, 31.3%), no retinal fluid (12/208, 5.8%) and patients whose data could not be assessed (12/208, 5.8%). The unavailable data resulted from data transfers following device software upgrading and changes in information technology.
There was no significant difference in VA and CMT value changes across the 5 fluid type groups over 5 year period after adjusting the baseline VA and CMT (P=0.901 and 0.573 respectively).

3.3.6 - Baseline choroidal neovascularisation type and treatment outcomes

Baseline FFA images were available on 170 of 208 (81.7%) patients at the time of image grading. Images that were lost resulted from data transfers following device software upgrading and changes in information technology. The majority of CNV type were occult followed by minimally classic and predominant classic (18.8% and 5.3%) respectively. There was no statistical significant difference in VA change across CNV types over the 5-year study period (p = 0.910). In analysing the relationship of CMT and baseline CNV types, no significant difference in CMT change over 5-years was found across CNV types (p = 0.160).

3.3.7 - Number of injections

The average number of injections per year was 7.5 (±2.6), 5.8 (±3.9), 6.4 (±4.5), 5.6 (±4.5) and 5.8 (±4.7) for years 1, 2, 3, 4 and 5, respectively. The average number of injections over 5-years was 30.8 (±17.4). Total number of injections was positively related with the change in VA over 5-years (p<0.0005) after adjusting for age and baseline VA (Table 7). The number of injections required by patients in each year of follow-up is expressed as a proportion of the total cohort (Figure 34) 36% of patients did not require an injection in their 5th year of follow-up, whilst 24% of patients required near monthly (10-12) injections in the 5th year.
Figure 33 - The proportions of all patients (n=208) in each year that required no injection, 1-3, 4-6, 7-9 or 10-12 injections.
3.3.8 - Adverse events

The most frequent ocular AEs were floaters (17.8%), eye pain (17.8%) and epiphora (6.3%). Ten patients (4.8%) had elevated IOP (>30mm Hg), which was controlled with topical anti-glaucoma medication. There were no cases of endophthalmitis, retinal tear, retinal detachment or lens trauma observed (Table 8).

Falls occurred in 22.6% of patients during the 5-years. Myocardial infarction, transient ischaemic attack, cerebral vascular accident (CVA) and coronary artery disease (CAD) were reported in 3.8%, 2.4%, 1.9% and 1.0% of patients, respectively. Other SAEs including angina, cancer and infections were low (all <0.5%) (Table 9).
Table 8 - Ocular adverse and serious adverse events in patients receiving ranibizumab therapy over a 5-year period (n=208)

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of Patients* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ocular Adverse Events</strong></td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td>37 (17.79)</td>
</tr>
<tr>
<td>Floaters</td>
<td>37 (17.79)</td>
</tr>
<tr>
<td>Epiphora</td>
<td>13 (6.25)</td>
</tr>
<tr>
<td>Elevated Intraocular Pressure</td>
<td>10 (4.81)</td>
</tr>
<tr>
<td>Subconjunctival Haemorrhage</td>
<td>10 (4.81)</td>
</tr>
<tr>
<td>Flashes</td>
<td>9 (4.33)</td>
</tr>
<tr>
<td>Discharge from Eye</td>
<td>3 (1.44)</td>
</tr>
<tr>
<td>Lid Swelling</td>
<td>2 (0.96)</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>2 (0.96)</td>
</tr>
<tr>
<td>Chalazion (study eye)</td>
<td>1 (0.48)</td>
</tr>
<tr>
<td>Ectropion (study eye)</td>
<td>1 (0.48)</td>
</tr>
<tr>
<td>YAG (both eyes)</td>
<td>1 (0.48)</td>
</tr>
<tr>
<td><strong>Ocular Severe Adverse Events</strong></td>
<td>19 (8.64)</td>
</tr>
<tr>
<td>Cataract Surgery</td>
<td>8 (3.84)</td>
</tr>
<tr>
<td>Vitreous Haemorrhage</td>
<td>2 (0.96)</td>
</tr>
<tr>
<td>SRH (causing VA reduction)</td>
<td>2 (0.96)</td>
</tr>
<tr>
<td>VMTS (study eye)</td>
<td>1 (0.48)</td>
</tr>
<tr>
<td>Corneal Abrasion</td>
<td>1 (0.48)</td>
</tr>
<tr>
<td>Corneal Abscess</td>
<td>1 (0.48)</td>
</tr>
<tr>
<td>Macular Hole</td>
<td>1 (0.48)</td>
</tr>
<tr>
<td>Recurrent Eye Infections</td>
<td>1 (0.48)</td>
</tr>
<tr>
<td>Ptosis (study eye)</td>
<td>1 (0.48)</td>
</tr>
</tbody>
</table>

*Several patients had multiple adverse events
YAG = Yttrium Aluminium Garnet Laser
SRH = Sub-retinal Haemorrhage
VMTS = Vitreo-Macular Traction Syndrome
Table 9 - Non-ocular adverse and serious adverse events in patients receiving ranibizumab therapy over a 5-year period (n=208)

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-Ocular Adverse Events</strong></td>
<td></td>
</tr>
<tr>
<td>Falls</td>
<td>47 (22.6)</td>
</tr>
<tr>
<td>Headaches and Nausea</td>
<td>1 (0.48)</td>
</tr>
<tr>
<td><strong>Non-Ocular Severe Adverse Events</strong></td>
<td></td>
</tr>
<tr>
<td>Myocardial Infarct</td>
<td>8 (3.84)</td>
</tr>
<tr>
<td>Transient Ischaemic Attack</td>
<td>5 (2.40)</td>
</tr>
<tr>
<td>Stroke</td>
<td>4 (1.92)</td>
</tr>
<tr>
<td>Coronary Artery Disease</td>
<td>2 (0.96)</td>
</tr>
<tr>
<td>Cardiac/Heart Failure</td>
<td>1 (0.48)</td>
</tr>
<tr>
<td>Angina</td>
<td>1 (0.48)</td>
</tr>
<tr>
<td>Valve Replacement</td>
<td>1 (0.48)</td>
</tr>
<tr>
<td>Atrial Fibrillation</td>
<td>1 (0.48)</td>
</tr>
<tr>
<td>CABG</td>
<td>1 (0.48)</td>
</tr>
<tr>
<td><strong>CABG = Coronary Artery Bypass Grafting</strong></td>
<td></td>
</tr>
</tbody>
</table>
3.4 - DISCUSSION

3.4.1 - Differences in patient characteristics between real-world cohorts and clinical trials

A baseline VA of greater than 6/12 (Snellen) was found in 40 (19%) patients in the LTRS cohort. Clinical trials often exclude patients with vision > 6/12. This limits possible VA gains due to a “ceiling” effect. In addition, this study included eyes that had received previous treatment for nAMD, and eyes with broader lesion types such as pigment epithelial detachment and extensive haemorrhages, which were excluded from some trials. (17, 77) The inclusion of these patients makes direct comparison with other clinical trial results difficult, however they represent the spectrum of disease treated in a real clinic setting.

3.4.2 - Long-term mean visual acuity

The majority of patients (79.8%) in this study cohort improved or maintained their vision over 5-years (losing ≤15 letters compared to baseline). The maximal average VA gain of 3.2 letters was observed after the first 6-months and slightly declined to approximately 2.0 letters at the end of one year. The results are consistent with previous long-term findings from clinic-based anti-VEGF therapy. (153, 154) The proportions of stable vision (losing <15 letters) and visual gain (gain >15 letters) at 1 year was less than the 1 year results in clinical trials. (17, 118) The differences in the degree of improvement are likely due to the differences in the baseline characteristics between trial patients and those in a real world setting as outlined in Chapter 3.4.1.
3.4.3 - The relationship between baseline visual acuity and 5-year visual acuity

Patients who had poor baseline VA showed a statistically significant improvement in VA while patients with good baseline VA trended towards a decline in their vision over 5-years (Table 7). This is consistent with the report of Pushpoth et al, (154) but contrasts with Rasmussen’s study, which found that patients with a baseline VA <6/30 Snellen had stable vision over 4 years. (158) Patients with VA < 6/60 may have very little potential for further measurable visual loss, exhibiting a “floor” effect of low VA change. Approximately 20% of patients with baseline Snellen VA better than 6/12 did not have the potential to improve their measured VA by >15 letters (“ceiling effect”). Of these, six patients with baseline VA in normal range had the greatest VA decline over the five years although the decrease was not statistically significant, likely due to the small sample size. The difference between overall mean VA and the VA outcomes adjusted by baseline VA may suggest that mean VA results from whole cohort on both previous treatment naïve and non-treatment naïve patients sometimes may mask good and poor responders to treatment.
3.4.4 - The relationship between the number of injections and 5-year visual acuity

A positive relationship was observed between number of injections and 5-year visual change. This equates to an increase of 0.3 letters in the 5-year VA for every increase of 1 injection (Table 7). This association is consistent with findings that more frequent injections produce better visual outcomes. (172, 173) However, in this cohort, the mean total number of injections over five years was 30.8, which is approximately half of the number injections that would be administered under a monthly regime. This finding demonstrates that vision may be maintained with less than monthly injections if regular review and assessment of disease activity is conducted.

3.4.5 - Anatomical outcomes

Previous studies investigating the response of fluid subtypes to ranibizumab therapy have shown that changes in SRF correspond with BCVA changes (174, 175). Recent studies have shown that at baseline, SRF is a favourable prognostic marker of visual outcomes, whereas IRF is a poor prognostic marker of the same. (140) In the LTRS cohort, neither fluid subtype was more responsive to ranibizumab therapy following long-term treatment. It is possible that there was no difference between fluid types was found in this cohort, as only a small proportion of patients had SRF at baseline (9 patients) meaning that a true difference would have been difficult to detect.

Some previous studies have demonstrated a relationship between vision and CMT (129) (175). Others found no correlation, especially when the SRF and IRF components were not separated in the measurement. (174) There was no correlation between CMT and 5-year VA in this study, possibly due to fact that when CMT was measured, any present SRF was included in the value.
3.4.5.1 - Challenges with accounting for several optical coherence tomography machines

There is little published data regarding the CMT changes in retrospective long-term studies, most likely due to difficulties in measurement and data collection. Central macular thickness was measured with different OCT devices during follow-up due to technology advances from TD-OCT to SD-OCT, which reflects changes that occurred in retinal clinics during this period. (176) Previous studies have showed that TD-OCT and SD-OCT are not interchangeable as Stratus OCT measures the thickness of the retina as the distance between the inner limiting membrane (ILM) and the IS/OS junction of the photoreceptors, Cirrus SD-OCT measures from the anterior border of the retinal pigment epithelium (RPE) to the ILM, while Spectralis OCT measures the distance from the posterior border of the RPE to the ILM. (177) Therefore, Spectralis OCT gives higher readings of macular thickness than Cirrus and Stratus OCT. (166, 169) This study covered a 5-year period. During the baseline period, only Stratus OCT was available. This was upgraded to SD-OCT shortly after the technology became available. Assessments were switched over to the higher resolution scanners with increased potential to detect fluid. As with most other study protocols, OCT examinations used for retreatment decisions utilised the currently available OCT device. In early randomised AMD clinical studies such as the Comparison of AMD Treatment Trials (CATT) and Prospective OCT Study with Lucentis for Neovascular AMD (PrONTO) TD-OCT was used. (129, 132) The CATT study used Stratus TD-OCT in the first year then changed over to Cirrus SD-OCT for 22.6% of scans in the second year. (118) (176) The retreatment decision in these trials was based on the presence of fluid in sub-retina detected by Stratus OCT including fast macular thickness map protocol. (118, 176) To compare the measurements from TD-OCT and SD-OCT a number of algorithms may be used. Bland and Altman devised a method of evaluating agreement between measurements of two devices by plotting their difference against their mean. The
measurements can be used interchangeably when the 95% CI of agreement is within a clinically acceptable range. (178) Hatef calculated retinal thickness from different devices by defining an internal and external retinal layer position using the intrinsic retinal segmentation algorithms in each device then averaged retinal thickness within nine retinal subfields in a 6 mm diameter circle centred on the fovea. (170) By manually measuring paired CMT on TD-OCT and SD-OCT obtained from the same patient, CATT study reported the absolute measurement difference was 25 µm or less in 71% of paired scans between two devices. (176)

In this study, 4 patients whose CMT was measured with Spectralis OCT at the 5 year assessment were excluded to avoid the possible significant differences and inconsistency of CMT measurements between Cirrus OCT and Spectralis OCT. In addition, measures were taken to reduce any possible variability by manually correcting segmentations and centralisation of fovea as needed. Based on the situation that the Stratus device records lower CMT values than Cirrus, the reduction in CMT seen with ranibizumab treatment in this study likely demonstrates a true finding as the trend of the Stratus machine to obtain lower CMT values would generally lead to an underestimation of any difference over 5-years by reducing the baseline value.
3.4.6 - Adverse events

Systemic SAE frequency for vascular thrombotic events, such as CVA was similar to other reported findings. (136) The total AE rate in this study was lower than for other published clinical trials. (118) Falls have not been previously reported in a retrospective long-term ranibizumab study. The LTRS found that falls occurred in 22.6% patients over 5 year period, giving an average yearly fall rate of 4.4%. This is similar to the 4.9% 1-year fall rate in the ranibizumab arm of the VEGF Trap-Eye: Investigation of Efficacy and Safety in Wet AMD (VIEW) study (136).

The LTRS found a low risk of serious ocular adverse events, comparable to the literature (endophthalmitis, with a quoted rate of 0.028%, or 1/3544 injections in a meta-analysis). (179) A high proportion of LTRS patients (24%) received 10-12 injections per month in the 5th year of treatment. Therefore, this population of patients would receive approximately 60 injections over 5-years, and statistically 1/59 of these patients would suffer from endophthalmitis (a potentially blinding condition, 3544/60 = 59.06) over 5-years. This extrapolated statistic could be considered to be more alarming than quoting a rate of 1/3544 injections, and helps frame the risk of treatment into context when considering the risk/benefit equation of treatment.

The relatively low SAE rate in the LTRS may relate to fewer injections during the longer follow-up period. The single treating physician in a single study centre with an established standard practice protocol for the patient assessment, treatment in an operating theatre setting and post treatment phone call follow-up may also have contributed to the lower rate of AEs and SAEs. (167, 180, 181)
3.4.7 - Strengths and limitations of the study

The strength of this retrospective study lies in the carefully designed protocol with strict inclusion and exclusion criteria, data collection time points, standardised re-treatment criteria and consistent clinical assessment and treatment by a single physician. This study differs from other published retrospective long-term ranibizumab studies, as it has analysed a considerably large number of participants (208) all with a 5-year follow-up with no more than a 6-month break between visits.

The major limitation of this study is its retrospective design, therefore increasing the amount of missing data. Other limitations of the study include difficulties reporting cataract progression due to non-standardised lens grading at each visit and the use of multiple OCT devices, which may have impacted on data accuracy. Although justifications of the CMT values between different OCT machines have been made in this study, variability of these measurements may still remain. In addition, the ICGA screening for PCV was not performed regularly at the baseline. The patients who were not responsive to treatment may potentially have included PCV patients.
3.5 - Conclusions

Ranibizumab is an effective therapy for nAMD patient using a disease activity guided individualised treatment model, particularly for patients with poor baseline vision. It provides stable vision in the majority of nAMD patients over 5-years of treatment. It is a safe long-term therapy, with relatively low AE rates. A greater number of injections are associated with improved visual outcomes, suggesting that regular treatment is of benefit even beyond the initial phase of treatment.
CHAPTER FOUR - Bilateral and sequential
neovascular age-related macular degeneration:
clinical presentation and treatment

4.1 - Background

Second eye nAMD disease is common, and its onset is often unpredictable and silent. (42) Prior to the widespread availability of OCT, clinicians were alerted to the possible presence of nAMD by visual symptoms, VA decline, or haemorrhage and exudation identified at the posterior pole with biomicroscopy. (63) Patients generally presented for ophthalmic assessment only when visual symptoms were noticed. (58)

During scheduled visits to assess the clinical response and progress of the first treated eye, opportunities exist to detect nAMD in the second eye. Most nAMD trials have excluded the data of second treated eyes, (182) therefore the clinical presentation and treatment outcomes of choroidal neovascularisation in second eyes is limited.. Furthermore, there is limited population based data assessing the role of AMD second eye screening. (183) The Amsler grid has been used as a screening tool for decades, but its ability to detect visual disease has remained in doubt in several studies (51) and it may not be a sufficiently sensitive instrument for monitoring vision. (52, 53)

The purpose of this study is to assess a cohort of patients with bilateral nAMD in whom the second eye was diagnosed with nAMD whilst the first eye was undergoing ranibizumab IVT, and therefore regular OCT imaging. VA outcomes, and OCT fluid characteristics were compared between first and second treated eyes, and presenting clinical features of the second eye were assessed.
4.2 - Methods

4.2.1 - Study design

The study population comprised a retrospective case series of all patients who commenced unilateral treatment with ranibizumab for nAMD at a tertiary referral retinal clinic between July 2007 and March 2012. Patients who subsequently commenced ranibizumab treatment in their second eye, with at least 12-months of follow-up for both eyes by a single ophthalmologist were included in the study.

Exclusion criteria included prior treatment in either eye with ranibizumab, bevacizumab or triamcinolone, vitrectomised eyes, PDT, focal laser or cataract surgery during the duration of the follow-up period.

4.2.2 - Second eye assessment

Patients being treated for nAMD in their first eye attended the clinic for assessment prior to each intravitreal injection. At each visit, measurements of Snellen VA using habitual correction and pinhole, and OCT examination was performed for both eyes, with the second eye OCT closely examined for the development of nAMD. New IRF or SRF detected by OCT posed an indication for FFA, which confirmed the presence of CNV in the second eye. Further details regarding patient assessment are in Chapter 2.

4.2.3 - Treatment protocol, optical coherence tomography analysis and fundus fluorescein angiography grading and statistical methods

As described in the general methods (Chapter 2)
4.3 - Results

A total of 45 patients satisfied the inclusion criteria. The mean time between diagnosis of second eye disease and previous clinic visit was 47.6 ±19.8 days. Baseline characteristics and injection characteristics of these patients are presented in Table 1. All patients underwent FFA imaging to confirm nAMD, however 7 cases could not be adequately graded retrospectively.
Table 10 - Baseline and injection characteristics of patients in the second eye ranibizumab study (n=45)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD) or Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General</strong></td>
<td></td>
</tr>
<tr>
<td>Age at first eye diagnosis (years)</td>
<td>79.4 (6.5)†</td>
</tr>
<tr>
<td>Age at second eye diagnosis (years)</td>
<td>80.7 (6.5)†</td>
</tr>
<tr>
<td>Gender – male</td>
<td>18/45 (40)</td>
</tr>
<tr>
<td>First treated eye – right eye / left eye</td>
<td>27/45 (60)</td>
</tr>
<tr>
<td>Baseline Visual Acuity first treated eyes (letters)</td>
<td>56.0 (20.6)†</td>
</tr>
<tr>
<td>Baseline Visual Acuity second treated eyes (letters)</td>
<td>62.2 (20.7)†</td>
</tr>
<tr>
<td>Second treated eye being the better seeing eye at baseline</td>
<td>29/45 (64.4)</td>
</tr>
<tr>
<td><strong>FFA Characteristics of first treated eyes</strong></td>
<td></td>
</tr>
<tr>
<td>Occult lesion</td>
<td>34/43 (79.1)‡</td>
</tr>
<tr>
<td>Classic lesion</td>
<td>9/43 (20.9)‡</td>
</tr>
<tr>
<td>Lesion Size</td>
<td>4.68mm² (4.6) †</td>
</tr>
<tr>
<td><strong>FFA Characteristics of second treated eyes</strong></td>
<td></td>
</tr>
<tr>
<td>Occult lesion</td>
<td>33/38 (86.87)‡</td>
</tr>
<tr>
<td>Classic lesion</td>
<td>5/38 (13.2)‡</td>
</tr>
<tr>
<td>Lesion Size</td>
<td>3.97mm² (4.7) †</td>
</tr>
<tr>
<td><strong>Time from last review to second eye diagnosis</strong></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>47.6 days (19.8) †</td>
</tr>
<tr>
<td><strong>Number of injections over first 12-months of treatment</strong></td>
<td></td>
</tr>
<tr>
<td>First treated eyes</td>
<td>7.47 (2.63)†</td>
</tr>
<tr>
<td>Second treated eyes</td>
<td>7.69 (3.0)†</td>
</tr>
</tbody>
</table>

SD = Standard Deviation  
FFA = Fundus Fluorescein Angiography  
*Mean (SD)  
‡Missing participant data (FFA scans not accessible for analysis (2 first eyes, 7 second eyes) All of these patients had IRF or SRF visible at baseline)
4.3.1 - Clinical profile of second treated eyes at diagnosis

Fifty-three per cent (24/45) of patients with second eye nAMD were asymptomatic on presentation (Figure 1). Eighteen patients of the total cohort (40%) were asymptomatic and also did not demonstrate a reduction in VA compared to their previous visit. Thirty-eight per cent (17/45) of patients had a reduction in VA of ≥5 letters from their previous visit.

Amongst the patients who were symptomatic in the second eyes (47%, 21/45), the most common symptom was “blurred vision” (62%) followed by metamorphopsia (24%). Of these symptomatic patients, 48% (10/21) also had a VA reduction of ≥5 ETDRS letters compared to the previous visit.

Intra-retinal fluid and/or sub-retinal fluid detected with OCT were the only clinical findings in 62% (15/24) of asymptomatic patients at diagnosis of second eye nAMD, and 33% (15/45) of all patients diagnosed with second eye disease. An illustrative example of an asymptomatic patient’s imaging and outcomes is shown in Figure 36.
Second-treated Eye nAMD
n = 45

Symptomatic
n = 21

Blurred Vision
n = 13

Metamorphopsia
n = 5

Scotoma
n = 2

Floaters
n = 1

Decreased VA and IRF/SRF
n = 4

SRF only
n = 1

Decreased VA and SRF
n = 1

SRF only
n = 1

SRF
n = 1

VA decreased
n = 6

IRF/IRF only
n = 6

No OCT Change
n = 1

IRF
n = 5

SRF
n = 1

Asymptomatic
n = 24

Heme on fundoscopy
n = 2

VA decreased + IRF/IRF
n = 6

IRF / SRF
n = 15

Missing Data
n = 1

Legend
nAMD = neovascular age-related macular degeneration
OCT = optical coherence tomography
IRF = intra-retinal fluid on OCT
SRF = sub-retinal fluid on OCT
Decreased VA = reduction ≥ 5 letters from previous visit

Figure 35 - Second treated eye clinical presentation: Optical coherence tomography characteristics at baseline and indications for diagnostic fundus fluorescein angiography study
Figure 36 - Images of the second treated eye of an asymptomatic patient presenting nAMD at routine follow-up of first treated eye undergoing ranibizumab injections. Second eye VA was 85 letters (Snellen VA 6/6) at baseline. Spectral-Domain OCT of the second eye at the previous visit, 28 days prior to baseline. (A) OCT at baseline; an aligned image to the previous visit with the arrow indicating new SRF. (B) 2 month OCT image aligned to baseline showing positive response following 2x monthly injections of ranibizumab, with the SRF no longer visible. (C) Aligned OCT image at 12-months, showing no SRF and flattening of pigment epithelial detachments seen at baseline and 2 months. VA at 12-months was 83 letters (Snellen VA 6/6.7). (D) Mid-venous phase fluorescein angiography at baseline (E), with a late phase fluorescein angiography showing an increased area of hyperfluorescence involving the foveal region consistent with an occult (Type 1) choroidal neovascular membrane. (F)
4.3.2 – Optical coherence tomography characteristics

Analysis of qualitative OCT changes (IRF/SRF) was restricted to the 37 patients who had a full set of OCT data at baseline and 12-month for both first and second eyes, with the remaining 8 patients having ungradable OCT images that were of poor quality in at least one of these 4 data collection points. One asymptomatic, second eye had a baseline OCT image that was not gradable (Figure 35).

Presence of Intra-retinal Fluid and Sub-retinal Fluid:
In second treated eyes, IRF was present in 54% of cases at baseline compared with 84% in first treated eyes (p=0.01). At 12-months, IRF was present in 22% of second treated eyes compared with 54% of first treated eyes (p<0.01) (Table 11). Sub-retinal fluid was present in 49% of cases of second treated eyes at baseline compared with 41% in first treated eyes (p=0.64). At 12-months, SRF was present in 14% of second treated eyes compared with 6% of first treated eyes (p<0.01) (Table 11).

Fluid free status after treatment:
In eyes with IRF or SRF visible on baseline OCT, second treated eyes had a higher fluid free state (no visible IRF or SRF) compared with first eyes, following 12-months of treatment (70% vs. 41%, p=0.02, Table 11).

Central macular thickness:
Mean CMT of second eyes was 50 µm lower than first eyes at baseline (p=0.03). Mean CMT of first treated eyes decreased by 76µm (from 307µm to 221µm) after 12-months compared to baseline (p=<0.001). Second treated eyes decreased by 24µm (from 257µm to 225µm) after 12-months compared to baseline (p=1.0).
Table 11 – Optical coherence tomography characteristics at baseline and 12-months post treatment comparing the same cohort of patients.

<table>
<thead>
<tr>
<th>OCT Characteristic (n=37*)</th>
<th>Baseline</th>
<th>12-months post treatment</th>
<th>p-value</th>
<th>Baseline</th>
<th>12-months post treatment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First eye</td>
<td>Second eye</td>
<td>p-value</td>
<td>First eye</td>
<td>Second eye</td>
<td>p-value</td>
</tr>
<tr>
<td>IRF</td>
<td>84% (31/37)</td>
<td>54% (20/37)</td>
<td>0.01</td>
<td>54% (20/37)</td>
<td>22% (8/37)</td>
<td>0.01</td>
</tr>
<tr>
<td>SRF</td>
<td>41% (15/37)</td>
<td>49% (18/37)</td>
<td>0.64</td>
<td>6% (5/37)</td>
<td>14% (5/37)</td>
<td>1</td>
</tr>
<tr>
<td>Fluid Free</td>
<td>0% (0/37)</td>
<td>8% (3/37)</td>
<td>0.24</td>
<td>41% (15/37)</td>
<td>70% (26/37)</td>
<td>0.02</td>
</tr>
<tr>
<td>CMT (μm)</td>
<td>307 (SD +/- 77)</td>
<td>257 (SD +/- 61)</td>
<td>0.03</td>
<td>221 (SD +/- 76)</td>
<td>225 (SD +/- 44)</td>
<td>0.76</td>
</tr>
</tbody>
</table>

*8/45 patients had an incomplete OCT data set, and therefore excluded from this analysis
- At baseline, nine first eyes and four second eyes had both IRF and SRF on OCT
- At 12-months post treatment, three first eyes and two second eyes had both IRF and SRF on OCT
4.3.3 - Visual acuity at baseline and at 12-months post ranibizumab therapy

The mean baseline VA in the first eye was 56.1 ±20.6 letters, compared with 62.2 ±20.2 letters in the second eye of the same patient (Figure 37), representing a difference of 6.1 letters (p=0.12). At 12-months following treatment, mean VA in the first eye improved by 1.7 letters compared to baseline although not statistically significant (p=0.49) and mean second eye VA increased 0.7 letters compared to baseline (p=0.69). There was no significant difference in mean VA change between the first eye and second eye in the same patient over the 12-month follow-up period (1.0 letters difference, p=0.36).

Second eyes had a higher rate of visual stability compared to first eyes if their baseline VA was of >76 letters (Snellen 6/9). Eighty-two per (9/11) cent of second eyes maintained VA of >76 letters at 12-months compared with 12% (1/8) of first eyes (p=0.05, Table 3). Baseline mean VA in this subgroup was 81.9 ±2.76 letters for the second eye vs. 79.0 ±2.44 letters for the first eye (p=0.03). The 12-month mean VA of second eyes in this subgroup was 77.4 ±6.2 letters compared to 64.2 ±13.2 letters in first eyes (p=0.02, Table 12).
Figure 37 - Aligned mean visual acuity letter scores of the first treated eye and second treated eye of the same patient over the first 12-months of ranibizumab therapy in each eye (n=45)
Table 12 – Visual acuity stability and optical coherence tomography findings between first and second eyes at 12-months, according to subgroups with good baseline visual acuity

<table>
<thead>
<tr>
<th>VA Subgroup</th>
<th>Treatment eye</th>
<th>n</th>
<th>Baseline mean VA letters (±SD)</th>
<th>12 month mean VA letters (±SD)</th>
<th>Proportion of eyes maintaining ≥76 letters at 12-months (%)</th>
<th>P value (Proportion of eyes maintaining ≥76 letters at 12-months compared to baseline, First eye vs. Second eye)</th>
<th>P value of first eyes vs. second eyes mean VA at baseline</th>
<th>P value of first eyes vs. second eyes mean VA at 12-months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline VA ≥76 letters (Snellen 6/9)</td>
<td>First eye</td>
<td>8</td>
<td>78.97 (2.45)</td>
<td>64.24 (13.17)</td>
<td>1/8 (12)</td>
<td>0.05</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Second eye</td>
<td>11</td>
<td>81.87 (2.76)</td>
<td>77.38 (8.01)</td>
<td>9/11 (82)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.4 - Discussion

4.4.1 - Symptoms and visual acuity change of the second eye at baseline

There is a high rate of second eye involvement in nAMD, and it may present as a bilateral, sequential disorder. (42) This study demonstrates that symptoms alone cannot be relied upon to indicate early second eye nAMD, with 53% of patients in our cohort being asymptomatic at diagnosis, which is higher than the 21.5% reported by Chevereaud et al. (184) Neurological compensation mechanisms can result in patients being asymptomatic in the early stages of nAMD (62) and previous histopathological studies have identified CNV membranes in asymptomatic patients. (66) Home detection devices for macular disease, including preferential hyperacuity visual field testing and hand-held OCT scanners (185) (186) are being trialled, but are not yet employed as standard practice.

Choroidal neovascularisation may exist for some time causing macular structural damage before becoming symptomatic or clinically detectable. (186) This emphasises the importance and utility of performing OCT to monitor the second eye of patients undergoing treatment for unilateral nAMD during their routine follow up visits in the clinic. Many patients (62%) developed nAMD in the second eye without an observable change in VA compared to the previous visit (62%). VA decrease of ≥5 letters was used as a marker of CNV activity and helped guide retreatment decisions in pro re nata (PRN) and treat and extend protocols of the PrONTO and SUSTAIN studies. (129, 187) In this study the most prevalent clinical finding was the presence of IRF and/or SRF imaged with OCT, and in 33% of patients, this was the only clinical indicator of nAMD in the second eye at baseline. Previous studies have shown that OCT fluid is a reliable marker of active nAMD (188) and OCT changes can be sudden and unpredictable. (189)
4.4.2 – Visual acuity change of second eyes over 12-months

Second eyes with good baseline acuity (>76 letters, or 6/9 Snellen VA) showed both a higher rate of maintenance of good VA and a higher mean VA at 12-months compared to first eyes, implying that early detection and treatment can help maintain vision (Table 12). This finding is consistent with a study by Ying et al which reported that a better VA at baseline correlated with a better final VA after treatment (190), suggesting that patients starting with a worse VA have an element of irreversible visual loss that cannot be rescued or stabilised with anti-VEGF therapy. Delay in anti-VEGF therapy for CNV could result in the ingrowth of fibrocytes, leading to permanent loss of retinal tissue and function within a short period of time (186) in the form of disciform scarring, the most common outcome of AMD related CNV.

4.4.3 - Anatomical outcomes of second eyes compared to first eyes

The proportion of second eyes that were fluid free at 12-months (70%) was higher compared to the CATT study groups which were given ranibizumab monthly (43% fluid free at 12-months). (132) This finding is more significant when considering the higher proportion of cases with fluid at baseline in this cohort compared to CATT (92% in this study vs. 71% in CATT) and the fewer average number of injections in this cohort. Furthermore, a higher rate of fluid resolution in second eyes compared to first eyes was observed in the same cohort of patients (70% vs. 41%, p=0.02) suggesting that early intervention can reduce the time taken to achieve a fluid free fovea, which may result in less irreversible structural damage to the retina. Recent guidelines suggest implementing a position of no-tolerance for OCT fluid (63) highlighting the importance of this reduced exudation. IRF in particular has been identified as a key baseline OCT feature indicating chronicity of CNV, and conferring poorer visual outcomes. (140) The rate of baseline IRF in this cohort was consistent with other reports (191, 192) with the second eyes demonstrating a statistically
significant lower rate at baseline and 12-months compared to first eyes (p=0.01, p=0.08). Early detection of nAMD in second eyes likely occurred in this cohort, thereby reducing the amount of exudative IRF occurring secondary to breakdown of the outer blood-retinal barrier (140), and subsequent irreversible retinal damage. This potentially explains the superior outcomes of second eyes in this cohort with good baseline VA at 12-months compared to first eyes.
4.4.4 - Study strengths and limitations

The strengths of this study lie in the standardised diagnosis, treatment and regular follow up including OCT imaging at each attendance with a single physician at a single treatment centre. The regular monitoring of both eyes in this cohort with a mean of 47.6 ±19.8 days between visits prior to diagnosis of second eye nAMD may have reduced the delay between development and detection of second eye disease unlike other studies, which have a greater interval between data collection points. Assessing the outcomes of first eye and second eye disease in the same cohort of patients reduced the effect of heterogeneity in patient response to treatment. Selecting a cohort treated by a single physician reduced the potential effect of different diagnosis and management decisions. Furthermore, the data relating to symptomatology of second eye disease has previously been given limited attention in the literature.

Limitations of this study include the small sample size, however the inclusion criteria were narrow by design to examine the difference of first vs. second eye outcomes in the same patient. The technology of the OCT scanner was upgraded during the study period, which may have influenced the ability to detect fluid. The image resolution of the scans was improved, therefore the high rate of fluid-free patients at 12-months is likely a reliable observation and more significant, as any fluid initially detected with a lower resolution TD-OCT scan would be seen on a higher resolution SD-OCT image.
4.5 - Conclusions

This study demonstrates that a high proportion of second eyes with angiographically diagnosed nAMD are asymptomatic, with OCT providing the only clinical indicator of nAMD. Reliance on symptoms, VA change and fundus examination as screening tools alone may delay detection and treatment of nAMD. Early detection and treatment of second eye nAMD in patients with good VA may potentially result in better visual outcomes. This may be especially critical for the second eye when it is the better seeing eye.
CHAPTER FIVE - General discussions and conclusions

5.1 - Key findings and implications of the thesis research

5.1.1 - Long-term ranibizumab study

The LTRS has investigated and examined the 5-year visual, anatomical and safety outcomes of ranibizumab used to treat nAMD in a real world clinic setting. This cohort is more representative of the patient population encountered in routine clinical practice compared to clinical trials with more restricted inclusion criteria and follow-up protocols, and the follow-up period is much longer than most clinical trials, which are usually designed to test the efficacy of a new therapy or treatment regime.

Ranibizumab treatment provided stability of VA for the majority of patients over 5-years, (79.8% lost <15 letters) with an average of 30.8 injections. Eleven per cent maintained a >15 letter improvement compared to baseline at 5-years.

There was significant variation in the number of injections required in the 5th year, with 36% of patients not requiring an injection, and 24% requiring 10-12 injections. This data therefore provides useful information for clinicians when counselling patients on the number of injections they will likely require in the long-term, and the real world visual outcomes. It also highlights that a large proportion of patients will require burdensome, regular long-term treatment, and also have greater exposure to the risks of treatment.

The published LTRS paper (134) was the first to describe 5-year outcomes in a real-world setting. To date, it remains one of the few “real world” studies published from
Australia, and is unlike other long-term studies (153, 154) in that it included a population of all patients who did continue treatment and monitoring for the entire 5-years, providing an insight into the outcomes of a population with demonstrated long-term compliance to the management of nAMD. The publication arising from the LTRS has been cited in a review of “real world outcomes”, (193) with the LTRS main visual acuity findings consistent with other studies. Although a disease guided, variable interval treatment model yielded clinically significant improvements in VA at all time points, they were inferior to clinical trials at 2-years, and that as a cohort, initial mean visual gains seen in the first 2-years are not maintained in the long-term. (193, 194) However, the mean VA change masks the findings that the majority of patients in the LTRS (79.2%) maintained their vision over 5-years (losing ≤15 letters). Another explanation for the inferior visual outcomes is that the real-world cohort included patients with Snellen VA >6/12 (meaning that the potential for VA gain was less, (ceiling effect of VA*), compared to most clinical trials.
5.1.2 - Second eye ranibizumab study

This study showed that over 50% of patients are asymptomatic at the time of diagnosis of nAMD in the second eye whilst being regularly monitored with OCT scanning.

In patients with good baseline visual acuity (>76 letters), visual outcomes were better in patient’s second treated eyes compared to their first, likely due to comparatively earlier detection and treatment (mean VA second eyes 77.38 ±6.2 letters vs. 64.24 ±13.2 letters at 12-months, p= 0.02) resulting in superior anatomical outcomes.

Relying on symptoms, VA, and fundus examination as screening tools for early nAMD potentially may result in many treatable cases of subfoveal nAMD being missed. Patients presenting with symptoms due to nAMD are likely to have already suffered irreversible visual loss. (58, 60) Optical coherence tomography may provide the only clinical finding indicating the presence of nAMD. This is one of few studies to assess the symptomatic status of nAMD patients with treatable CNV lesions, and the results provide a cautionary tale to clinicians who advise patients to only present for care of they become symptomatic. Furthermore, it follows that with this finding: the second eyes of patients should undergo opportunistic OCT scans at every visit whilst attending for treatment in the first eye.
Patient’s second eyes also demonstrated superior anatomical outcomes. Fifty-four per cent of second eyes had IRF at baseline compared with 83.7% of first eyes (p=0.01). IRF has been identified as a marker of occult CNV chronicity. (140) The OCT fluid free rate of second eyes at 12-months was 70% vs. 41% in first eyes (p=0.02). To the candidate’s knowledge, this is the first study to assess the anatomical outcomes of second eye disease in the same cohort of patients detected whilst undergoing treatment for the first eye. The study design helped to address inter-patient variability of treatment adherence and response to therapy.
5.2 - Challenges and limitations

5.2.1 – Optical coherence tomography evolution of technology

Due to developments in OCT imaging technology from time to spectral domain, SRC upgraded devices in the clinic to a Cirrus Spectral-Domain OCT system. This provided far superior imaging resolution, with the low-resolution Stratus system capturing 768 A-scans in a 6mm “star” arrangement vs. the Cirrus system capturing 65536 A-scans in a 6mm cube. This transition to SD-OCT therefore reflected the adoption of improved imaging technology likely to confer clinical benefit for patient care secondary to improved image resolution, occurring in most ophthalmic practices world-wide. Although unavoidable, this change presented challenges in performing comparisons between each of the machines for research purposes, affecting patients who started on the TD-OCT and progressed to the SD-OCT. The anatomical boundaries measured are different between these machines, meaning that the CMT values from the SD-OCT would be higher than TD-OCT (see Chapter 3.4.5.1). Since our results showed a significant CMT reduction between baseline scans (TD-OCT) and 5-year scans (SD-OCT) we did not perform a conversion, as it would not have changed the overall study finding. Several nAMD clinical trials also made the same transition from TD-OCT to a SD-OCT system mid-study, including the CATT study. (176)
5.2.2 - Retrospective design and missing data

Given that both LTRS and SERS were retrospective case series’, there was inevitable missing data compared to a prospective study. This did not affect the visual acuity data or the safety data, as these records were complete. Missing data was most common with OCT images, where the quality of some scans was too poor to contribute to the study. In the LTRS, 25 baseline OCT images were of too poor a quality to grade qualitative findings of IRF and/or SRF. In the SERS, 8/45 patients had at least one image that were not gradable in the first or second eye at baseline or 12-months, therefore the OCT analysis was restricted to the remaining 37 patients as it was critical to have a complete data set to analyse qualitative findings over time.

Baseline FFA images were unavailable for grading in 18.2% (38/208) patient in the LTRS and 6.6% (3/45) of the SERS, as they were performed on a FFA camera where the images were no longer accessible at the time of the studies. This did not materially impact on the key findings of the studies, as this data included the size and CNV lesion type, the analysis of which was not a primary aim of the thesis. Despite this data not being available, all patients commencing ranibizumab treatment had the appropriate FFA study at the time of diagnosis, confirming subfoveal nAMD. This is a requirement to access government subsidised ranibizumab (this criteria is outlined in Chapter 2).
5.3 - Future directions for research

There remain unanswered questions regarding the indications for cessation of ranibizumab therapy once there is no active nAMD detected and the method and frequency of follow-up. Furthermore, the rate of recurrence of nAMD following the cessation of treatment is also unknown, along with the degree of irreversible visual loss following a recurrence. Reliable predictors for successful cessation of therapy would be clinically meaningful.

Baseline biomarkers for treatment response are also limited, although there is some emerging evidence that qualitative OCT findings can predict likely disease course and final visual outcomes. (140) The presence of SRF has been associated with a relatively benign disease course compared to IRF, and may be the best candidates for TaE regimes. (140) Prospective studies are needed to validate this idea.

There is a need for research into the most effective and cost effective means for detecting new disease in the second eye, and recurrence in the first-treated eye. Emerging technologies in the form of home-based hyperacuity devices and handheld OCT’s may provide an alternative means for monitoring for recurrence, and second eye disease. Alternatively, a collaborative OCT screening program in conjunction with other eye-care professionals (e.g. optometrists) may help facilitate early detection and thus optimise visual outcomes for asymptomatic patients.
5.4 - Conclusions of this thesis

Ranibizumab is an effective treatment, with minimal serious ocular adverse events, or systemic serious adverse events when used in a real-world clinical setting over 5-years. This suggests that it can be used in the long-term for treatment of nAMD and other vascular retinal diseases.

The individualised treatment regime resulted in stabilisation of VA in a large proportion of patients at 5-years. This regime is similar to TaE, and is less burdensome than a monthly or PRN treatment protocol. The VA results were inferior to clinical trials at the 1 and 2 year time points, consistent with other reports on real-world outcomes. Likely reasons include a reduced injection number, and a heterogeneous patient group, including patients with a baseline VA higher than clinical trial patients. Despite this, the individualised regime has merit over a monthly or PRN regime given the vastly reduced number of injection, lessening the burden on patients, carers and health systems.

Optical coherence tomography scans of both eyes at all visits to an eye-care professional may be warranted, as it can detect signs of nAMD prior to other clinical indicators. Second eye disease often occurs with no symptoms or change in VA. The maximal time delay between OCT evidence of nAMD and irreversible damage is not known, however early detection and treatment of second eye nAMD leads to superior outcomes at 1-year. This reduces the burden of visual loss to patients, carers and the health system.
APPENDICES
Long-term ranibizumab study baseline data collection sheet

☐ Signed informed consent:

Baseline
Study ID:
Data Collector:

Which eye was initiated on Ranibizumab treatment on this visit?
Right / Left Eye / Both

Was the second eye initiated on Ranibizumab at a later clinic visit?  Y / N
If yes, complete “second eye baseline form”

Baseline visit date: / / 

General information
Date of birth: / /  Gender: M / F

Medical history
☐ Smoker / Ex smoker / Non Smoker / Unknown
☐ Relevant Ocular conditions/procedures during follow-up period: Y / N

<table>
<thead>
<tr>
<th>Right (Date)</th>
<th>Left (Date)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cataract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cataract Surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YAG capsulotomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other maculopathy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Appendix A: Long-term ranibizumab study baseline collection form Page 1/3
Relevant AMD treatment prior to follow-up period: Y / N

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RIGHT (Y/N)</th>
<th>LEFT (Y/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal laser (for AMD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avastin:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Vitrectomy in study eye: Y / N Date: ___________

Relevant Medical history: Y / N

<table>
<thead>
<tr>
<th></th>
<th>BL or Date of onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular disease</td>
<td></td>
</tr>
<tr>
<td>(CABG, Stent, AMI)</td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
</tr>
<tr>
<td>DVT / PE / Other Blood clot event</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td></td>
</tr>
</tbody>
</table>

Eye Exams Baseline Date

<table>
<thead>
<tr>
<th></th>
<th>RIGHT</th>
<th>LEFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCVA (Snellen)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IOP (mmHg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Appendix A: Long-term ranibizumab study baseline collection form Page 2/3
OCT machine (Baseline) | Stratus | Cirrus | Spectralis | Not Recorded
---|---|---|---|---
FA Date: | | | | |
Fundus Photo Date: | | | | |

<table>
<thead>
<tr>
<th>OCT Image:</th>
<th>RIGHT</th>
<th>LEFT</th>
<th>NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRF / PED / Cystic / Fibrosis</td>
<td>SRF / PED / Cystic / Fibrosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central retinal thickness:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNV type:</td>
<td>Classic / Occult / Mixed</td>
<td>Classic / Occult / Mixed</td>
<td></td>
</tr>
<tr>
<td>Lesion size (mm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fundus Photo:</td>
<td>Haem / Lipid</td>
<td>Haem / Lipid</td>
<td></td>
</tr>
</tbody>
</table>

Appendix A: Long-term ranibizumab study baseline collection form Page 3/3
# Long-term ranibizumab study - follow-up data collection sheet

<table>
<thead>
<tr>
<th>Study Eye: RIGHT / LEFT</th>
<th>Study Baseline Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Month</td>
<td></td>
</tr>
<tr>
<td>Date of Visit:</td>
<td>1/1/1</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>RIGHT</th>
<th>LEFT</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCVA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Thickness</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Stratus</th>
<th>Cirrus</th>
<th>Spectralis</th>
<th>Not Recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|                      | 3 Month |        |            |              |
|                      | 1/1/1   |        |            |              |

<table>
<thead>
<tr>
<th></th>
<th>RIGHT</th>
<th>LEFT</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCVA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Thickness</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Stratus</th>
<th>Cirrus</th>
<th>Spectralis</th>
<th>Not Recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|                      | 6 Month |        |            |              |
|                      | 1/1/1   |        |            |              |

<table>
<thead>
<tr>
<th></th>
<th>RIGHT</th>
<th>LEFT</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCVA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Thickness</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Stratus</th>
<th>Cirrus</th>
<th>Spectralis</th>
<th>Not Recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

Appendix B: Long-term ranibizumab study follow-up data collection form Page 1/4
### 12 Month

<table>
<thead>
<tr>
<th>Date of Visit: <em><strong>/</strong></em>/___</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>BCVA</th>
<th>CENTRAL THICKNESS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>STRATUS</th>
<th>CIRRUS</th>
<th>SPECTRALIS</th>
<th>NOT RECORDED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 18 Month

<table>
<thead>
<tr>
<th>Date of Visit: <em><strong>/</strong></em>/___</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>BCVA</th>
<th>CENTRAL THICKNESS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>STRATUS</th>
<th>CIRRUS</th>
<th>SPECTRALIS</th>
<th>NOT RECORDED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 24 Month

<table>
<thead>
<tr>
<th>Date of Visit: <em><strong>/</strong></em>/___</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>BCVA</th>
<th>CENTRAL THICKNESS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>STRATUS</th>
<th>CIRRUS</th>
<th>SPECTRALIS</th>
<th>NOT RECORDED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Appendix B: Long-term ranibizumab study follow-up data collection form Page 2/4
### 30 Month

- **Date of Visit:** __/__/___

<table>
<thead>
<tr>
<th></th>
<th>RIGHT</th>
<th>LEFT</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCVA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Thickness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratus</td>
<td>Cirrus</td>
<td>Spectralis</td>
<td>Not Recorded</td>
</tr>
</tbody>
</table>

### 36 Month

- **Date of Visit:** __/__/___

<table>
<thead>
<tr>
<th></th>
<th>RIGHT</th>
<th>LEFT</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCVA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Thickness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratus</td>
<td>Cirrus</td>
<td>Spectralis</td>
<td>Not Recorded</td>
</tr>
</tbody>
</table>

### 42 Month

- **Date of Visit:** __/__/___

<table>
<thead>
<tr>
<th></th>
<th>RIGHT</th>
<th>LEFT</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCVA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Thickness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratus</td>
<td>Cirrus</td>
<td>Spectralis</td>
<td>Not Recorded</td>
</tr>
</tbody>
</table>

Appendix B: Long-term ranibizumab study follow-up data collection form Page 3/4
### 54 Month

<table>
<thead>
<tr>
<th></th>
<th>RIGHT</th>
<th>LEFT</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BCVA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Central Thickness</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Stratus</th>
<th>Cirrus</th>
<th>Spectralis</th>
<th>Not Recorded</th>
</tr>
</thead>
</table>

### 60 Month

<table>
<thead>
<tr>
<th></th>
<th>RIGHT</th>
<th>LEFT</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BCVA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Central Thickness</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Stratus</th>
<th>Cirrus</th>
<th>Spectralis</th>
<th>Not Recorded</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Year 4</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of Visits for AMD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Number of Ranibizumab Injections in study eye</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Appendix B: Long-term ranibizumab study follow-up data collection form Page 4/4
Long-term ranibizumab study – adverse events data collection sheet

*Only record ocular adverse events in the Study Eye

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Y / N</th>
<th>Date</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floaters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sub-conjunctival Haemorrhage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lid Swelling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grittiness / Foreign Body Sensation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irritation to prep</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epiphora</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑IOP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endophthalmitis,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitreous Haemorrhage,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinal Tear,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinal Detachment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Falls</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Appendix C: Long-term ranibizumab study adverse events collection form Page 1/1
Long-term ranibizumab study – OCT grading data collection sheet

<table>
<thead>
<tr>
<th>Patient Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study ID</td>
</tr>
<tr>
<td>Grader</td>
</tr>
<tr>
<td>Grading Date</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Image Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gradable</td>
</tr>
<tr>
<td>Forced Grading</td>
</tr>
<tr>
<td>Ungradable</td>
</tr>
<tr>
<td>Not Present</td>
</tr>
</tbody>
</table>

OCT Machine

- Stratus 0
- Cirrus 1
- Spectralis 2
- Other ______

1. OCT Data

<table>
<thead>
<tr>
<th>Central Macular Thickness (μm)</th>
<th>Volume (mm²)</th>
<th>Comments</th>
</tr>
</thead>
</table>

2. Intra-retinal Fluid (IRF)

| Absent                          | 0            |
| Questionable                    | 1            |
| Present                         | 2            |
| Outside Central                 | 3            |
| Central Point Involved          | 4            |
| CG                              | 8            |
| IRF Thickness at Centre Point (μm) |              |
| IRF Max Thickness Along Any Scan (μm) |              |

3. Sub-retinal Fluid (SRF)

| Absent                          | 0            |
| Questionable                    | 1            |
| Present                         | 2            |
| Outside Central                 | 3            |
| Central Point Involved          | 4            |
| CG                              | 8            |
| SRF Thickness at Centre Point (μm) |              |
| SRF Max Thickness Along Any Scan (μm) |              |

Appendix D: LTRS and SERS OCT data collection form Page 1/1
Second eye ranibizumab study baseline data collection sheet

**Second eye which underwent Ranibizumab treatment:**

Right / Left Eye

**Baseline visit date for initiation of Lucentis in the second eye:** / /

<table>
<thead>
<tr>
<th>Eye Exams</th>
<th>RIGHT</th>
<th>LEFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptom duration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCVA (Snellen)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IOP (mmHg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fundus Photo, FA and OCT Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCT machine (Baseline)</td>
</tr>
<tr>
<td>FA Date:</td>
</tr>
<tr>
<td>Fundus Photo Date:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>OCT Image:</th>
<th>RIGHT</th>
<th>LEFT</th>
<th>NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRF / PED / Cystic / Fibrosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRF / PED / Cystic / Fibrosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central retinal thickness:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNV type:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classic / Occult / Mixed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classic / Occult / Mixed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesion size (mm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fundus Photo:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haem / Lipid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haem / Lipid</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Appendix E: Second eye ranibizumab study baseline collection form Page 1/**
Second eye ranibizumab study follow-up data collection sheet

Study Eye: RIGHT / LEFT

Study Baseline Date:

1 Month

- Date of Visit: __ / __ / __

<table>
<thead>
<tr>
<th></th>
<th>RIGHT</th>
<th>LEFT</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCVA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Thickness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratus</td>
<td>Cirrus</td>
<td>Spectralis</td>
<td>Not Recorded</td>
</tr>
</tbody>
</table>

3 Month

- Date of Visit: __ / __ / __

<table>
<thead>
<tr>
<th></th>
<th>RIGHT</th>
<th>LEFT</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCVA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Thickness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratus</td>
<td>Cirrus</td>
<td>Spectralis</td>
<td>Not Recorded</td>
</tr>
</tbody>
</table>

6 Month

- Date of Visit: __ / __ / __

<table>
<thead>
<tr>
<th></th>
<th>RIGHT</th>
<th>LEFT</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCVA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Thickness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratus</td>
<td>Cirrus</td>
<td>Spectralis</td>
<td>Not Recorded</td>
</tr>
</tbody>
</table>
12 Month

- Date of Visit: ___ / ___ / ___

<table>
<thead>
<tr>
<th></th>
<th>RIGHT</th>
<th>LEFT</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCVA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Thickness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratus</td>
<td>Cirrus</td>
<td>Spectralis</td>
<td>Not Recorded</td>
</tr>
</tbody>
</table>

Number of Ranibizumab Injections in the second eye
REFERENCES

41. 'Eyes on the future - A clear outlook on age-related macular degeneration'.


123. Ferrara N, Damico L, Shams N, Lowman H, Kim R. Development of ranibizumab, an anti-vascular endothelial growth factor antigen binding fragment, as


154. Pushpoth S, Sykakis E, Merchant K, Browning AC, Gupta R, Talks SJ. Measuring the benefit of 4 years of intravitreal ranibizumab treatment for neovascular


