Bevacizumab in a treat-and-extend regimen for neovascular age related macular degeneration in a resource limited sector

by

Dr Etienne Philip Le Roux

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University of KwaZulu-Natal

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Declaration

I, Dr E.P. Le Roux, declare that:

(i) The research reported in this dissertation, except where otherwise indicated, is my original work.

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Overview of the thesis

Age-related macular degeneration (AMD) is the leading cause of visual loss in people over 50 years of age in developed countries. AMD is a degenerative disorder affecting the macula and is characterized by the presence of specific clinical findings. Conventionally AMD is divided into two main types, dry (non-exudative) AMD and wet (exudative/neovascular) AMD. Dry AMD is the most common form with geographic atrophy (GA) a sign of advanced stage of disease. Neovascular AMD is associated with more rapid progression to advanced sight loss but is less common.

For neovascular AMD (nAMD) the standard of care at present are inhibitors of vascular endothelial growth factor (anti-VEGF), of which there are three currently on the market. Bevacizumab (trade name Avastin) is a recombinant humanized monoclonal antibody that blocks angiogenesis by inhibiting vascular endothelial growth factor-A, and is used off-label for nAMD. Ranibizumab (trade name Lucentis) is a monoclonal antibody fragment (Fab) created from the same parent mouse antibody as bevacizumab and has U.S. Food and Drug Administration (FDA) approval for the treatment of neovascular AMD. Aflibercept (trade name Eylea) is a soluble decoy receptor that binds vascular endothelial growth factor-A and placental growth factor-And also has FDA approval for the treatment of nAMD. Not only do the anti-VEGF drugs maintain vision they also improve vision which was not possible before their introduction onto the market around 2007, which was a major step forward in the management of nAMD patients.

Bevacizumab is by far much more cost effective option as it is marketed for certain metastatic cancers including metastatic colon cancer which was approved by the FDA in 2004. Using only one vial, one can treat at least twenty patients with nAMD. The intravitreal injection is prepared in compounding pharmacies from the vial of bevacizumab. Bevacizumab has been proven to be as effective and safe as Ranibizumab in major prospective randomized clinical trials and in the resource limited public health sector bevacizumab is the drug of choice for nAMD.

The purpose of the study was to determine if the clinical outcomes of patients using intravitreal bevacizumab with a treat-and-extend regimen (TER) for nAMD resulted in and maintained the same visual gains as a monthly regimen does as was determined in major prospective randomized clinical trials. The benefit of a TER is that it can drastically decrease the number of clinic visits and the number of injections received by patients. In a resource limited sector, such as the public health sector, this would drastically save costs and decrease the burden on the health practitioners as well as the whole public health system when managing these patients.

At present there are three treatment regimen options for nAMD using bevacizumab: Fixed monthly injection regimen (FMR), *pro re nata* (PRN) regime with monthly follow-up, and TER.

FMR is the regimen adopted in the initial major landmark trials. Patients are seen every month and injected every month. This regimen has proven to give the best visual outcome and is seen as the gold standard. Many different dosing options have been tried in multiple clinical trials to attempt to decrease the burden on the health sector. Quarterly dosing, injecting every three months, and PRN with less than monthly follow-up were shown to be inferior to fixed monthly. PRN regime with monthly follow-up was shown to have results similar to fixed monthly but was still inferior and very importantly still requires patients to be seen monthly. TER is an alternative type of PRN regime that entails administering three initial injections at monthly intervals and then to continue monthly injections until the retina is dry as determined using optical coherence tomography (OCT). After the retina is dry the period between injections are gradually increased by 2 weeks at a time and continued injections at every visit regardless if retina is dry or not. If fluid accumulates again the period between injections is decreased by 2 weeks at a time until a stable interval is determined for each individual patient. This regimen cuts down on clinic visits and the number of injections and maintains vision according to results from past and ongoing clinical trials.

A retrospective chart review was undertaken of all patients with nAMD that presented to Inkosi Albert Luthuli Central Hospital (IALCH) since records were kept. 53 eyes of 48 patients met the entry criteria from June 2011 until June 2016. The main outcome measures were: mean change from baseline visual acuity at year 1 and 2, mean change from baseline central macular thickness at year 1 and 2, mean number of intravitreal bevacizumab injections at year 1 and 2 and mean period of extension between intravitreal bevacizumab injections.

In reviewing the charts it was ascertained that patient's vision did indeed improve significantly from baseline at year 1 and 2. It was noticed during review of the charts that many patients did not follow an absolute and strict TER, either due to poor follow-up due to social circumstances, such as finance or transport issues, or clinician error when deciding on intravitreal injection frequency. This resulted in a mixture of TER and PRN regimen being followed in many patients, which could reveal a more real world experience.

This study has shown that our current treatment for nAMD is resulting in significant visual gains but our implementation of the TER can be followed more strictly. Areas identified where intervention would make a difference include, education of treating Ophthalmologists, especially the junior registrar's and medical officers, on the TER protocol, educating patient on the importance of follow-up on correct clinic appointments and possible down referring patients back to base ophthalmology clinics with the adequate staff and equipment for management and follow-up. This would be much more convenient for patients as IALCH

ophthalmology clinic is one of only two clinics in Kwa-Zulu-Natal currently providing this service and many patients travel a great distance for their clinic appointments.

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Part 1: The Review of Literature

Introduction

Age-related macular degeneration (AMD) is the leading cause of visual loss in people over 50 years of age in developed countries [1]. AMD is a degenerative disorder predominantly affecting the macula. [6]

AMD is divided into two main types, dry (non-exudative) AMD and wet (exudative/neovascular) AMD. [2] Dry AMD is the most common form with geographic atrophy (GA) a sign of advanced stage of disease.

Neovascuar AMD (nAMD) only found in 10% to 20% of patients with AMD but accounts for 90% of vision loss from AMD and is associated with more rapid progression to advanced vision loss.[7] The main manifestations of nAMD are choroidal neovascularization (CNV). CNV are new abnormal blood vessels that grow in the choroid and break through the barrier between the choroid and the retina into the sub-retinal pigment epithelium (type 1) or subretinal (type 2) space. [2] Terminology used to describe CNV on fundus fluorescein angiography (FFA) was derived from the Macular Photocoagulation Study. Classic CNV fills with dye in a well-defined 'lacy' pattern during early transit of the dye. With Occult CNV the limits cannot be fully defined on FA. Predominantly or minimally classic CNV is defined as the classic element of the lesion on FFA is greater or less than 50% of the total lesion. [2] Untreated CNV forms scar tissue that eventually replaces normal retina tissue, causing irreversible visual loss.

The aetiology of AMD is not fully understood. Genetic risk factors and environmental risk factors, such as smoking, may contribute to the development and progression of AMD. Smoking roughly doubles the risk of AMD[1] while genetic factors, such as complement factor H gene and complement component 3 genes, are consistent with an inflammatory basis for the development of AMD .[6] Age is another major risk factor. AMD is also more common in Caucasians than other races. Hypertension, high fat intake and obesity, cataract surgery, blue iris colour, high sunlight exposure, and female gender are suspected. [2]

Treatment for dry AMD at intermediate or advanced stage is high dose of micronutrient supplementation as determined by the Age-related Eye Disease Study 1 (AREDS 1) and Age-related Eye Disease Study 2 (AREDS 2)[7] In AREDS 1 progression to advanced AMD occurred in 28% of placebo group and 20% of micronutrient group, which included 500 mg vitamin C, 400 IU vitamin E, and 15 mg β– carotene, 80 mg Zn and 2 mg copper.[8] In AREDS 2 because of the

increased risk of lung cancer in smokers who take β-carotene, β-carotene was substituted by lutein and zeaxanthin. The evidence from AREDS 2 on the beneficial and adverse effects of substituting lutein and zeaxanthin for B-carotene suggests it could be more appropriate than β-carotene in AREDS-type supplements. [9] It is therefore suggested that current and previous smokers use the AREDS formula substituting lutein and zeaxanthin for β-carotene. Current management of dry AMD include annual surveillance of patients above 60 years and cessation of smoking. No evidence supports supplements for early dry AMD but patients with intermediate or advanced dry AMD should self-monitor vision at home with an Amsler grid and should take AREDS supplements. Therapies that inhibit complement and alter visual cycle are being tested and for severe disease stem cell therapy is being tested.[7]

When patients progress to advanced AMD with CNV under the fovea, they are classified as having nAMD. [1] For nAMD the standard of care at present are inhibitors of vascular endothelial growth factor (anti-VEGF), of which there are three currently on the market. Bevacizumab (trade name Avastin) is a recombinant humanized monoclonal antibody that blocks angiogenesis by inhibiting vascular endothelial growth factor-A, and is used off-label for nAMD. Ranibizumab (trade name Lucentis) is a monoclonal antibody fragment (Fab) created from the same parent mouse antibody as bevacizumab and has U.S. Food and Drug Administration (FDA) approval for the treatment of nAMD. Aflibercept (trade name Eylea) is a soluble decoy receptor that binds vascular endothelial growth factor-A and placental growth factor, and also has FDA approval for the treatment of nAMD.

Not only do the anti-VEGF drugs maintain vision they also improve vision which was not possible before their introduction onto the market around 2007, which was a major step forward in the management of nAMD patients.

History of the treatment of nAMD

Photocoagulation

The first effective treatment of CNV was thermal laser photocoagulation. In 1982 the Macular Photocoagulation Study Group reported that patients receiving argon laser treatment to extrafoveal and juxtafoveal CNV lesions had a decreased rate of visual decline compared to untreated control group. Note that because this treatment however caused irreversible damage to the overlying neurosensory retina it was not appropriate for subfoveal lesions. This treatment also had a recurrence rate of more than 50% [1].

Photodynamic therapy

Photodynamic therapy (PDT) introduced in 2000 offered an alternative to thermal laser photocoagulation. PDT selectively targets CNV without damaging the overlying retinal tissue. Verteporfin, an intravenously injected photosensitizing dye, preferentially accumulates within the neovascular tissue. Non-thermal laser light of 689nm for 83 seconds activates the verteporfin which forms free radicals causing endothelial damage and thrombosis of the choroidal vessels. [1] The Therapy of Age-related macular degeneration with Photodynamic therapy (TAP) Study Group conducted a randomized trial comparing PDT with placebo and analysed visual acuity loss between the 2 groups. At 2 years of follow-up after randomization, 53% of PDT patients lost 15 or fewer letters of visual acuity, compared to 38% of placebo patients which was statistically significant. PDT was however less effective in some CNV types and it was recommended that verteporfin therapy be used for the treatment of patients with subfoveal predominantly classic l CNV from nAMD. [11]

By 2000 we had two options for the treatment of CNV in nAMD: Thermal laser photocoagulation for extrafoveal and juxtafoveal CNV lesions and PDT for subfoveal predominantly classic l CNV. PDT is less destructive method of treatment compared to laser but was not associated with any visual acuity gain.[1] We also did not have effective treatment for subfoveal minimally classic and occult CNV.

Introduction of anti-vascular endothelial growth factor

The development of pharmacologic agents that specifically target angiogenesis, such as anti-VEGF drugs, has revolutionized the treatment of nAMD.[3] VEGF increases the growth of abnormal blood vessels and augments vascular permeability. VEGF is up-regulated by hypoxia, and its levels are increased in both the vitreous and retina of patients with retinal vascular disease.

Pegaptanib sodium (trade name Macugen) was the first drug directed against VEGF. Pegaptanib is an aptamer that directly binds to $VEGF_{165}$ and prevents it from activating DNA transcription. Pegaptanib is administered by intravitreal injection every six weeks. [1] The VEGF Inhibition Study In Ocular Neovascularization (VISION), studied patients with all angiographic choroidal neovascularization lesion compositions of nAMD and received either intravitreal pegaptanib sodium (0.3 mg, 1 mg, 3 mg) or sham injections every 6 weeks for 54 weeks. The results in the VISION trial showed that pegaptanib had a beneficial effect for all subtypes of neovascularisation. We now had an effective and safe treatment for subfoveal minimally classic and occult CNV as well .The majority of patients in the pegaptanib clinical trials still lost vision but at a slower rate, and the treatment is considered one that slows the disease rather than one that improves visual acuity.[10] Because newer more effective anti-VEGF soon followed, pegaptanib was no longer used for the treatment of CNV due to AMD.

Fixed monthly regimen

Ranibizumab (Lucentis) is a recombinant anti-VEGF monoclonal antibody fragment that is capable of inhibiting all active forms of VEGF in the eye. Two major studies have examined the effects of ranibizumab in eyes with nAMD. The phase III Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular Age-Related Macular Degeneration (MARINA) trial, and The Anti-VEGF Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization in Age-Related Macular Degeneration (ANCHOR) trial. MARINA compared ranibizumab with sham injection for treatment of minimally classic and occult nAMD. ANCHOR compared ranibizumab with PDT in patients with classic CNV. MARINA and ANCHOR trials were both 2‑year, multicentre, double blind, randomized control trials, and demonstrated that visual acuity outcomes when treating nAMD with fixed monthly regimen(FMR) of intravitreal ranibizumab was far superior to any other treatment to date. [4]

MARINA and ANCHOR trials demonstrated significant visual gain for the first time in the history of treatment of nAMD. Ranibizumab was licenced by the FDA in 2006 and in Europe in 2007.[6]

Less than monthly follow-up

Subsequent studies followed MARINA and ANCHOR which aimed at reducing frequency of injections. Monthly intravitreal injection is a major burden on patients and the health system. Alternative fewer fixed scheduled injections have not resulted in visual outcomes that were as favourable as fixed monthly injection**.**

Efficacy and safety of ranibizumab on subfoveal CNV with or without classic CNV secondary to AMD (PIER) trial showed quarterly dosing of ranibizumab after 3 monthly doses inferior to monthly dosing.[5] Efficacy and safety of monthly versus quarterly ranibizumab treatment in

neovascular age-related macular degeneration(EXCITE) trial also showed FMR to be superior to three monthly injections and then quarterly injections. [12]

The open-label extension trial of ranibizumab for choroidal neovascularization secondary to age-related macular degeneration (HORIZON) trial showed visual acuity decreased when patients from the MARINA and ANCHOR trial switched to PRN dosing followed 6 monthly then changed to 3 monthly follow-up. [13] Safety of Ranibizumab in Subjects with Neovascular Age-related Macular Degeneration (SAILOR) trial also showed FMR to be superior to *pro re nata* with less than monthly follow-up. [5]

Pro re nata (PRN) with monthly follow-up

Prospective optical coherence tomography imaging of patients with neovascular age-related macular degeneration treated with intraocular ranibizumab (PrONTO) study was the first prospective trial evaluating the use of ranibizumab in a *pro re nata* (PRN) approach with monthly follow-up for nAMD.

At 2 years, the mean visual acuity improved by 11.1 letters ($P < .001$) and the OCT-CRT decreased by 212 µm ($P < .001$). These VA and OCT outcomes were achieved with an average of 9.9 injections over 24 months. The visual acuity outcomes were similar to the landmark pivotal MARINA and ANCHOR trials while using fewer injections. [14] In the MARINA study at 2 years the 0.5mg ranibizumab group‘s mean visual acuity improved by 6.6 letters ($p<0.001$) and in ANCHOR study at 2 years the ranibizumab group’s mean visual acuity improved between 8.1 and 10.7 letters ($p<0.001$).[15]

The PrONTO study results lead to two more major clinical trials the comparison of AMD treatment trials (CATT) and the inhibit VEGF in age-related choroidal neovascularization (IVAN) trial. CATT and IVAN were designed to answer the question of which regimen was superior, FMR of anti-VEGF or PRN regimen of anti-VEGF with monthly follow-up. CATT and IVAN were also deigned to compare ranibizumab and bevacizumab.

Bevacizumab (Avastin) is an anti-VEGF monoclonal complete antibody and is very much cheaper than ranibizumab. It was approved by the FDA for treatment of metastatic colon cancer in 2004. The agent has been used off-label as a treatment option for nAMD having been shown to be as safe and effective in several retrospective and prospective studies including CATT and IVAN. Bevacizumab is by far much more cost effective option compared to ranibizumab and aflibercept, which was introduced later. Using only one vial of bevacizumab you can treat at least twenty patients with nAMD. The intravitreal injection is prepared in compounding pharmacies from the vial of bevacizumab.[1]

Fixed monthly versus *Pro re nata* with monthly follow-up.

CATT was a randomized, prospective, head-to-head, non-inferiority trial comparing ranibizumab to bevacizumab has shown that both anti-VEGF agents have equivalent effects on visual acuity when administered according to the same regime. [3]

At 1 year bevacizumab given monthly gained 8.0 letters, was as effective as ranibizumab given monthly with 8.5 letters gained. Bevacizumab given as PRN with monthly follow-up gained 5.9 letters and was as effective as ranibizumab given PRN with monthly follow-up with 6.8 letters gained.[16]

Ranibizumab given PRN with monthly follow-up was equal to ranibizumab monthly at 1 year but at 2 years monthly ranibizumab was superior to PRN with monthly follow-up with 2.4 letter difference between groups. At 2 years the gain in visual acuity from baseline was 8.8 letters in the ranibizumab group following a FMR and 7.8 letters in the bevacizumab given monthly group. In the ranibizumab PRN with monthly follow-up group it was 6.7 letters gained and 5.0 letters gained in the bevacizumab PRN with monthly follow-up group.[21]

Also switching from FMR to PRN treatment at the start of year 2 resulted in greater mean decrease in vision during year 2. Comparison between bevacizumab monthly and bevacizumab PRN with monthly follow-up was inconclusive. [15]

IVAN was a randomized, prospective, non-inferiority trial comparing ranibizumab to bevacizumab given either monthly or PRN with monthly follow-up. At 1 year comparison between ranibizumab and bevacizumab was inconclusive. FMR was equivalent to PRN with monthly follow-up. [15]

Importantly bevacizumab was equally as effective as ranibizumab in both CATT and IVAN.

VEGF trap-eye: Investigation of efficacy and safety in wet AMD (VIEW 1 and 2) compared monthly and every 2 monthly dosing of aflibercept with monthly ranibizumab. Aflibercept (Eylea) or VEGF Trap-Eye binds all forms of VEGF-A to D as well as Placental Growth Factor. VIEW 1 and 2 trials showed all doses of aflibercept dosing of 2mg every month, 0.5mg every month and 2mg every 2 months non-inferior to ranibizumab 0.5mg every month. [15]

To summarize monthly ranibizumab, bevacizumab or aflibercept give similar results. PRN with monthly follow-up is an option but was inferior to monthly treatment at 2 years in the CATT study and still requires monthly follow-up. Aflibercept 2 monthly may be an alternative to FMR ranibizumab and bevacizumab. Aflibercept remains extremely expensive and is not a treatment option for the public health sector.

As stated earlier, bevacizumab has been proven to be as effective and safe as ranibizumab in major prospective randomized clinical trials and in the resource limited public health sector bevacizumab is the drug of choice for nAMD.

Treatment regimen options for bevacizumab

At present there are three treatment regimen options for nAMD using bevacizumab: FMR, PRN regime with monthly follow-up and TER. FMR is the regimen adopted in the initial major trials. Patients are seen every month and injected every month. This regimen has proven to give the best visual outcome and is seen as the gold standard. Many different dosing options have been tried in multiple clinical trials to attempt to decrease the burden on the health sector. Quarterly dosing, injecting every three months, and PRN with less than monthly follow-up were shown to be inferior to FMR. PRN regime with monthly follow-up was shown to have results similar to fixed monthly but was still inferior and very importantly still requires patients to be seen monthly.

TER is an alternative type of PRN regime that entails administering three initial injections at monthly intervals and then to continue monthly injections until the retina is dry as determined using optical coherence tomography (OCT). After the retina is dry the period between injections are gradually increased by 2 weeks at a time and continued injections at every visit regardless if retina is dry or not. Continue to extend the interval, 12 weeks usually being the maximum interval period of extension. If fluid accumulates again, the period between injections is decreased by 2 weeks at a time until a stable interval is determined for each individual patient. This regimen cuts down clinic visits and reduces the number of injections while it maintains vision similar to FMR according to results from past and ongoing clinical trials.

Treat-and-extend versus PRN dosing

In a study by Oubraham et al, TER had better gain in letters compared to PRN regimen with a few more injections of 8 vs 5 in the PRN group.[5]

In a systemic review of as needed PRN regimen versus TER of ranibizumab or bevacizumab for nAMD, including 8 studies meeting the TER protocol and 62 studies meeting the PRN protocol were included. The mean visual acuity gain was 5.4 letters in the PRN group and 10.4 letters in the TER group. The PRN group received an average of 5.6 injections in year 1 and the TER group 8.09. Central retinal thickness improved on average by 100.3 μm in the PRN group compared with 87.7 μm in the TER group. [17]

Treat-and-extend versus fixed monthly dosing

Two studies by Gupta et al and Shienbaum et al employed a TER of ranibizumab and bevacizumab respectively for treatment of nAMD with favourable visual acuity results with significantly fewer visits and intravitreal injections compared with treatment in a FMR.[3 and 4]

In a prospective TER study published in Retina in 2014 the mean visual acuity change from baseline was +9.5 and +8.0 letters after 12 months and 24 months respectively with a mean of 8.6 injections in the first year and 5.6 in the second year.[18]

The CANTREAT trial (Canadian Treat and Extend Analysis Trial with Ranibizumab) is a multicentre, randomized trial designed to evaluate and compare a FMR of ranibizumab to the TER approach in achieving and maintaining a maximum visual acuity. It is theorized that the TER will be non-inferior to monthly dosing in terms of mean change in visual acuity from baseline to 1 year while decreasing the number of injections in the TER. It is expected that the TER approach will allow for proactive, individualized treatment compared to PRN reactive treatment, giving better results than PRN regimen in terms of visual acuity. [19]

Interim data from the CANTREAT trial showed the mean visual acuity gain was 6.8 letters in the FMR arm and 7.2 letters in the TER arm at 1 year. Patients in the FMR arm and TER arms received an average of 11.9 and 9.9 injections respectively at 12 months. [20]

What other benefits are there of fewer intravitreal injection of Anti-VEGF?

Endophthalmitis is a major risk factor with intravitreal injection with devastating visual consequences. Retrospective studies have reported that the incidence of post intravitreal injection endophthalmitis ranges from 0.02 to 1.6%. Multiple recent large meta-analysis reports indicate the incidence rates between 0.049 to 0.056%. Although the rate of endophthalmitis is low for any individual patient the potential incidence rises when receiving repeated monthly injections.[22]

It is a logical assumption if we can decrease the number of intravitreal injections needed by a patient we can decrease the risk of endophthalmitis.

Other ocular complications include non-infectious inflammation to the biologic anti-VEGF agents, retinal tears and detachment, retinal pigment epithelium rip, elevated intraocular pressure post intravitreal injection and cataract. [23]

Evidence suggesting that receiving more anti-VEGF injections is associated with geographic atrophy progression comes from numerous studies. Geographic atrophy was the main reason of decreased vision in patients receiving intravitreal anti-VEGF in the MARINA trial. [23] The SEVEN-UP study observed geographic atrophy progression in nAMD patients treated with ranibizumab over a mean of 7.4 years. The CATT trial found that patients treated with monthly anti-VEGF intravitreal injection had higher rate of geographic atrophy progression than the patients treated with the PRN regimen with monthly follow-up. [24]

Systemic safety profile of intravitreal anti-VEGF is not known to date with confounding evidence and persistently unanswered questions. As is well known, VEGF is a potent promoter of vascular hyper-permeability. Anti-VEGF agents significantly reduce vascular hyper-permeability, and when given systemically can raise systemic arterial blood pressure. Systemically delivered anti-VEGFs are known to promote the development of arterial thrombotic events but this has been observed at much larger doses than intravitreal injection of anti-VEGF. [23]

There have been no statistically significant evidence derived from the numerous studies that intravitreal injection of anti-VEGF leads to increased development of arterial thrombotic events however these clinical trials were not statistically powered to answer this question. Subgroup analysis and pharmacokinetic data points to the possibility of a risk of arterial thrombotic events from intravitreal anti-VEGF drugs. The populations at the highest risk for arterial thrombotic events based upon available clinical trial data, clinical experience and theoretical factors are diabetics, patients 85 years and older and patients with history of prior strokes. [23] It is therefore reasonable to want to decrease the number of intravitreal anti-VEGF injections and extend the period between intravitreal anti-VEGF injections.

Conclusion

While clinical trials are ongoing with novel therapies to decrease the number of office visits and intravitreal injections a TER has been shown in other clinical trials to be as effective as the gold standard of FMR and may be superior to PRN regime. TER reduces the number of office visits as well as the number of intravitreal injections and at the same time potentially decreasing the risk of complications and side-effect from intravitreal injection of anti-VEGF. Bevacizumab off label is far cheaper than ranibizumab and it is essential to investigate the visual outcomes and economic impact of this treatment strategy for nAMD in our resource limited setting. [3].

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Part 2: A submission ready manuscript.

Cover Page

Dr Etienne Philip Le Roux, Registrar UKZN, IALCH

800 Bellair Rd, Durban

0828550907

epleroux@yahoo.com

Dr Carl-Heinz Kruse, Head Of Department, Greys Hospital

Town Bush Rd, Pietermaritzburg

0840110767

ruraleye@gmail.com

Corresponding author: Dr Etienne Philip Le Roux.

Summary

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Bevacizumab in a treat-and-extend regimen for neovascular age related macular degeneration in a resource limited sector

Objective: To obtain the clinical outcomes of patients using intravitreal bevacizumab with a treat-and-extend regimen for neovascular age-related macular degeneration (nAMD) and determine if this regime will benefit resource limited sectors in managing these patients.

Design: A retrospective chart review of patients who received intravitreal bevacizumab for nAMD between June 2011 and June 2016.

Subjects: 53 eyes of 48 patients, 1 year follow-up (n=53) and 2 year follow-up (n=24).

Methods: Patients were diagnosed with nAMD using fundus fluorescein angiography and optical coherence tomography at the first visit. Patients initiated on treatment followed a treat-and-extend treatment protocol.

Main outcome measures: Main outcome measures was mean change from baseline visual acuity (VA) at year 1 and 2, mean change from baseline central retinal thickness (CRT) at year 1 and 2, mean number of intravitreal bevacizumab injections at year 1 and 2 and mean period of extension between intravitreal bevacizumab injections.

Results: Mean VA at start of treatment was 0.21 and improved to 0.38 year 1 and 0.45 year 2. The mean CRT at the start of the study was 333.46 µm and improved to 265.35 µm at year 1 and 245.79µm at year 2. The mean number of injections in year 1 was 9.64 and 6 in year 2. The mean number of weeks between injections year 1 was 5.59 and 8.58 in year 2.

Conclusion: In this study it was ascertained that patient's VA did improve significantly from baseline but our implementation of the treat-and-extend regimen can be followed more strictly.

Introduction

The current standard of care for neovascular age-related macular degeneration (nAMD) is intravitreal injection of an anti-vascular endothelial growth factor (anti-VEGF). Monthly injection places an enormous burden on the health system and on the patients themselves.

It has long been known that patients respond differently to anti-VEGF therapy and not all patients need monthly injection. In an attempt to decrease the burden on the health sector and patients many different treatment regimens have been tried and tested in clinical trials to decrease clinic visits as well as the number of injections received by patients with varying results.

Intravitreal injection of anti-VEGF is not without risk, such as the risk of endophthalmitis, serious adverse systemic events and evidence of progression of geographic atrophy with repeated injections.[1,2]

At present there are three treatment regimen options for nAMD using bevacizumab. Fixed monthly injection regimen (FMR), *Pro re nata* (PRN) regime with monthly follow-up, and treat-and-extend regimen (TER).

FMR was adopted in the initial major trials. Patients are seen every month and injected every month. This regimen has proven to give the best visual outcome and is seen as the gold standard. Many different dosing options have been tried in multiple clinical trials to attempt to decrease the burden on the health sector but were shown to be inferior to a FMR. PRN with monthly follow-up was shown to have results similar to FMR but was still inferior and very importantly still requires patients to be seen monthly. TER is an alternative type of PRN regime that entails administering three initial injections at monthly intervals and then to continue monthly injections until the retina is dry as determined using optical coherence tomography (OCT). After the retina is dry the period between injections are gradually increased by 2 weeks

at a time and continued injections at every visit regardless if retina is dry or not. Continue to extend the interval between injections, 12 weeks is usually the maximum interval period of extension. If fluid accumulates again the period between injections is decreased by 2 weeks at a time until a stable interval is determined for each individual patient.

The reason for this study was to determine the clinical outcomes of patients using intravitreal bevacizumab (BVZ) with a TER for nAMD and determine if this regime will benefit resource limited sectors in managing these patients. Evidence in the literature is growing that a TER is as effective in restoring and maintaining vision as the current gold standard of FMR and more effective than a PRN with monthly follow-up, with fewer injections and fewer office visits. Interim 1 year data from the Canadian Treat and Extend Analysis Trial with Ranibizumab (CANTREAT), showed the mean visual acuity gain was equivalent in the TER arm compared to the FMR despite fewer injections.[3]

A retrospective chart review was undertaken of all patients with nAMD that presented to Inkosi Albert Luthuli Central Hospital (IALCH) where electronic patient records were kept. 53 eyes of 48 patients met the entry criteria from June 2011 until June 2016. In this paper we are able to show that a TER may be a safe and cost-effective alternative to the current gold standard of fixed monthly injection of an anti-VEGF for nAMD adding to the growing body of evidence in favour of this treatment regimen.

Research methods and design

Patient Selection

Charts of all patients diagnosed with nAMD at IALCH during the period of June 2011 until June 2016 with at least 1 year follow-up were reviewed and included in this study.

The inclusion criteria included all patients with nAMD diagnosed using fluorescein angiography (FA) and optical coherence tomography (OCT) and treated according to the TER using the anti-VEGF bevacizumab.

All other causes of choroidal neovascularization such as dominant drusen, angioid streaks, polypoidal choroidal vasculopathy etc. were excluded from the patient cohort.

Fifty three eyes from forty eight patients, with fifty three having 1 year follow-up and twenty four having 2 year follow-up were included.

Visual acuity (VA) and central retinal thickness (CRT) was obtained at the first visit, at year 1 and at year 2. Total number of injections in year 1 and year 2 was obtained as well as the interval period between each injection.
Mean change from baseline VA at year 1 and 2, mean change from CRT at year 1 and 2, mean number of intravitreal bevacizumab injections in year 1 and 2 and mean period of between intravitreal bevacizumab injections was determined.

Statistical Analysis

Data were entered into Statistical Packages for the Social Sciences for analysis (SPSS). A one-sample t-test was used to assess the differences in VA at year 1 and year 2 and central retinal thickness at year 1 and year 2.

Ethical considerations

As this was a retrospective chart review, informed consent was not obtained from patients included in this study. No patient contact was made and site permission was obtained from Inkosi Albert Luthuli Hospital management. Institutional Review Board and Ethics committee approval was obtained from the University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC), BE017/15. The described research adhered to the Declaration of Helsinki.

Results

Of the 48 patients in the study more than 90% were above the age of 70y. [Table 1] Age is one of the most important risk factors for the development of nAMD.[4]

Age (years)	Frequency	Percent
< 70	4	7.5
70 - 74	9	17.0
75 - 79	13	24.5
80 - 84	16	30.2
≥ 85	11	20.8
Total	53	100.0

Table 1: Age

Of the 53 study eyes, 29 were from male patients.[Table 2]

Sex	Frequency	Percent
Female	24	45.3
Male	29	54.7
Total	53	100.0

Table 2: Sex

Race	Frequency	Percent
Black	3	5.7
Indian	8	15.1
White	42	79.2
Total	53	100.0

Table 3: Race

The majority of patients were of European descent. [Table 3] nAMD has a strong genetic association and is almost exclusively found in Caucasians.[4]

	N	Minimum	Maximum	Mean	Std. Deviation	Skewness	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error
VA_Start	53	0.01	0.80	0.21	0.174	1.637	0.327
VA_1year	53	0.10	1.00	0.38	0.267	0.846	0.327
VA_2year	24	0.10	1.00	0.45	0.266	0.519	0.472

Table 4: Visual Acuity

VA_Start = Visual acuity at the Start of Treatment.

VA_1year/2year = Visual Acuity at Year 1 and Year 2.

The visual acuity was recorded in the decimal system for ease of recording and statistical analysis. A VA of counting fingers was given a decimal score of 0.01. Data on VA at the start of treatment was available for 53 eyes, 53 eyes at year 1 and 24 eyes at year 2.

The best VA recorded was 1.0 and 0.01 the worst VA recorded. Mean VA at start of treatment was 0.21 and improved to 0.38 at year 1 and 0.45 at year 2.[Table 4] Final mean visual acuity converted to a visual acuity score (VAS) was done to be able to determine number of letters gained. Mean VAS at the start of treatment was 66, at year 1 was 79 with 13 letters gained and 83 at year 2 with 17 letters gained.

	N	Minimum	Maximum	Mean	Std. Deviation	Skewness	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error
CRT_Start	48	215.0	611.0	333.46	85.443	1.160	0.343
CRT_1year	48	134.0	774.0	265.35	93.555	3.528	0.343
CRT_2year	24	172.0	349.0	245.79	49.773	0.494	0.472

Table 5: Central Retinal Thickness

CRT_Start = Central Retinal Thickness at the Start of Treatment.

CRT_1/2 = Central Retinal Thickness at Year 1 and Year 2.

Central retinal thickness (CRT) was measured using optical coherence tomography (OCT). Data on central retinal thickness of 48 eyes was available at the start of treatment and 48 eyes at year 1 and 24 eyes at year 2. The mean CRT at start of the study was 333.46 μm and improved to 265.35 μm at year 1 and 245.79 μm at year 2. Table[5]

	Total Number of Injections	Number of Eyes	Mean Number of Injections
Year 1	511	53	9.64
Year 2	144	24	6

Table 6: Number of Injections

The mean number of injections in year 1 was 9.64 per eye and in year 2 the mean number of injections was 6 per eye.[Table 6]

The mean number of weeks between injections in year 1 was 5.59 and 8.58 in year 2. [Table 7]

	Mean Number of Weeks Between Injections	Std. Deviation
Year 1	5.59	1.18
Year 2	8.58	3.13

Table 7: Number of Weeks between Injections

Discussion

In this study it was ascertained that patient's vision did improve significantly from baseline at year 1 and 2. Central retinal thickness improved significantly at year 1 and year 2 and the average number of injections was less than with FMR and similar to other TER regimens.

Because this was a retrospective chart review a decimal system was used to record vision in the patients charts using a standard Snellen chart and this visual acuity was used in our study. Most, if not all studies conducted in the literature, uses the Early Treatment of Diabetic Retinopathy Study (ETDRS) chart and ETDRS protocol. This is considered to represent the gold standard for VA measurements.[5]

The ETDRS protocol was not followed in our study but to be able to compare our results with the landmark clinical trials we converted our final mean VA into a visual acuity score (VAS). With the VAS we are able to determine the number of letters gained in VA and compare our results to other trials where the VAS scale was calculated on ETDRS charts, where 1 point is credited for each letter read correctly.

The Minimally classic/occult trial of the anti VEGF antibody Ranibizumab in the treatment of Neovascular ARMD (MARINA) and Anti-VEGF antibody for the treatment of predominantly classic choroidal neovascularization in AMD (ANCHOR) trials were the landmark clinical trials and demonstrated significant visual gain for the first time in the history of treatment of nAMD. In MARINA and ANCHOR patients followed a FMR with injections every month for two years.

<u>Fixed Monthly Regimen</u>

In the MARINA study at 2 years the 0.5mg ranibizumab group's mean VA improved by 6.6 letters ($p<0.001$) and in ANCHOR study at 2 years the ranibizumab group's mean VA improved between 8.1 and 10.7 letters ($p<0.001$).[10].

Subsequent studies followed MARINA and ANCHOR which aimed at reducing frequency of injections. Monthly intravitreal injection is a major burden on patients and the health system.

Less than monthly follow-up

Alternative fewer fixed scheduled injections have not resulted in visual outcomes that were as favourable as a FMR.

Efficacy and safety of ranibizumab on subfoveal CNV with or without classic CNV secondary to AMD (PIER) trial showed quarterly dosing of ranibizumab after 3 monthly doses inferior to a FMR.[6]

Efficacy and safety of monthly versus quarterly ranibizumab treatment in neovascular age-related macular degeneration (EXCITE) trial also showed a FMR to be superior to three monthly injections and then quarterly injections. [12]

The open-label extension trial of ranibizumab for choroidal neovascularization secondary to age-related macular degeneration (HORIZON) trial showed VA decreased when patients from the MARINA and ANCHOR trial switched to PRN dosing. [13]

Safety of Ranibizumab in Subjects with Neovascular Age-related Macular Degeneration (SAILOR) trial also showed FMR to be superior than PRN with less-than-monthly follow-up.[6]

Pro re nata with monthly follow-up

Prospective optical coherence tomography imaging of patients with neovascular age-related macular degeneration treated with intraocular ranibizumab (PrONTO) study was the first prospective trial evaluating the use of ranibizumab in a PRN approach with monthly follow-up

for nAMD. At 2 years, the mean visual acuity improved by 11.1 letters ($P < .001$) and the OCT-CRT decreased by 212 µm ($P < .001$). These VA and OCT outcomes were achieved with an average of 9.9 injections over 24 months. The visual acuity outcomes were similar to the pivotal landmark MARINA and ANCHOR trials while using fewer injections. [14]

Fixed monthly versus *Pro re nata* with monthly follow-up

The PrONTO study results lead to two more major clinical trials, the comparison of AMD treatment trials (CATT) and the inhibit VEGF in age-related choroidal neovascularization (IVAN) trial.

CATT and IVAN were designed to answer the question of which regimen was superior, FMR or PRN with monthly follow-up. CATT and IVAN were also deigned to compare the therapeutic efficacy of the molecules ranibizumab and bevacizumab.

Bevacizumab (Avastin) is an anti-VEGF monoclonal complete antibody and is very much cheaper than ranibizumab. It was approved by the FDA for treatment of metastatic colon cancer in 2004. The agent has been used off-label as a treatment option for nAMD having been shown to be as safe and effective in several retrospective and prospective studies including CATT and IVAN. Bevacizumab is by far much more cost effective option; using only one vial of bevacizumab you can treat at least twenty patients with nAMD. The intravitreal injection is prepared in compounding pharmacies from the vial of bevacizumab.[4]

CATT was a randomized, prospective, head-to-head, non-inferiority trial comparing ranibizumab to bevacizumab has shown that both anti-VEGF agents have equivalent effects on visual acuity when administered according to the same regime. [8]

At 1 year bevacizumab given monthly gained 8.0 letters, was as effective as ranibizumab given monthly with 8.5 letters gained. Bevacizumab given as PRN with monthly follow-up gained 5.9

letters and was as effective as ranibizumab given PRN with monthly follow-up with 6.8 letters gained.[15]

Ranibizumab given PRN with monthly follow-up was equal to ranibizumab given with a FMR at 1 year but at 2 years monthly ranibizumab was superior to PRN with monthly follow-up with a 2.4 letter difference between groups.

At 2 years the gain in visual acuity from baseline was 8.8 letters in the ranibizumab given with a FMR and 7.8 letters in the bevacizumab given with a FMR. In the ranibizumab given PRN with monthly follow-up group it was 6.7 letters gained and 5.0 letters gained in the bevacizumab given PRN with monthly follow-up.[16] Also switching from a FMR to a PRN treatment regimen in year 2 resulted in greater mean decrease in vision during year 2. [10]

Treat-and-Extend: IALCH

In our study the mean VAS at the start of treatment was 66, at year 1 it was 79 with 13 letters gained and 83 at year 2 with 17 letters gained. The mean number of injections in year 1 was 9.64 per eye and in year 2 the mean number of injections was 6. The mean number of weeks between injections in year 1 was 5.59 and 8.58 in year 2. These results are very comparable to results in a prospective treat-and-extend study published in Retina in 2014.[11] The mean visual acuity change from baseline was 9.5 at year 1 and 8.0 letters at year 2 with a mean of 8.6 injections in year 1 and 5.6 injections in year 2 in this study.

Treat-and-extend versus *pro re nata* with monthly follow-up

In a study by Oubraham et al [6], a TER had better gain in letters compared to a PRN regimen with a few more injections of 8 in the TER group versus 5 in the PRN group.

In a systemic review meta-analysis of as needed PRN regimen versus TER's of ranibizumab or bevacizumab for nAMD, 8 studies met the TER protocol and 62 studies met the PRN protocol were included. The mean VA gain was 5.4 letters in the PRN group and 10.4 letters in the TER group in year 1. The PRN group received an average of 5.6 injections in year 1 and the TER group 8.09. This compared to our gain of 13 letters in year 1 and to our mean number of injections in year 1 of 9.64. Central retinal thickness improved on average by 100.3 μm in the PRN group compared with 87.7 μm in the TER group compared to our study of 68.1 μm at year 1. [7]

Treat-and-extend versus fixed monthly regimen

Two studies by Gupta et al and Shienbaum et al [8 and 9] employed a TER of ranibizumab and bevacizumab respectively for treatment of nAMD with favourable VA results with significantly fewer visits and intravitreal injections compared with treatment in a FMR.

The Canadian Treat and Extend Analysis Trial with Ranibizumab (CANTREAT) is the first multicentre, randomized trial designed to evaluate and compare head-to-head a FMR dosing of ranibizumab to the TER approach in achieving and maintaining maximum VA.

Interim data from the CANTREAT trial showed the mean visual acuity gain was 6.8 letters in the FMR dosing arm and 7.2 letters in the TER dosing arm at 1 year. Patients in the FMR dosing arm and TER dosing arms received an average of 11.9 and 9.9 injections respectively at 12 months. [3]

What other benefits of fewer intravitreal injection of Anti-VEGF?

Endophthalmitis is a major risk factor with intravitreal injection with devastating visual consequences. Retrospective studies have reported that the incidence of post intravitreal injection endophthalmitis ranges from 0.02 to 1.6 %. Recent large meta-analyses report the

incidence rates between 0.049 to 0.056 %. Although the rate of endophthalmitis is low for any individual patient the potential incidence rises when receiving repeated monthly injections.[17] Other ocular complications include non-infectious inflammation to the biologic anti-VEGF agents, retinal tears and detachment, retinal pigment epithelium rip, elevated intraocular pressure post intravitreal injection and cataract. [19]

Evidence suggesting that receiving more frequent anti-VEGF injections is associated with geographic atrophy progression comes from numerous studies. Geographic atrophy was the main reason of decreased vision in patients receiving intravitreal anti-VEGF in the MARINA trial.[18]

The SEVEN-UP study observed geographic atrophy progression in nAMD patients treated with ranibizumab over a mean of 7.4 years. The CATT trial found that patients treated with monthly anti-VEGF intravitreal injection had higher rate of geographic atrophy progression than the patients treated with the PRN with monthly follow-up regimen. [19]

Systemic safety profile of intravitreal anti-VEGF is not known to date with confounding evidence and persistently unanswered questions. As known VEGF is a potent promoter of vascular hyper-permeability. Anti-VEGF agents significantly reduce vascular hyper-permeability, and when given systemically can raise systemic arterial blood pressure. Systemically delivered anti-VEGF is known to promote the development of arterial thrombotic events but this has been observed at much larger doses than intravitreal injection of anti-VEGF. [19]

There have been no statistically significant evidence derived from the numerous studies that intravitreal injection of anti-VEGF leads to increased development of arterial thrombotic events, however these clinical trials were not statistically powered to answer this specific question. Subgroup analysis and pharmacokinetic data points to the possibility of a risk of arterial thrombotic events from intravitreal anti-VEGF drugs.

The populations at the highest risk for arterial thrombotic events based upon available clinical trial data, clinical experience and theoretical factors are diabetics, patients 85 years and older and patients with history of prior strokes. [19]

It is therefore reasonable to want to decrease the number of intravitreal anti-VEGF injections and extend the period between intravitreal anti-VEGF injections.

Limitations of the study

Being a retrospective chart review is a limitation in itself. It was also noticed while collecting the data that there was poor compliance with the TER protocol at IALCH, resulting in many patients not following an absolute and strict TER. One reason that contributed to this was most probable due to poor follow-up by patients due to social circumstances, such as finance or transport issues. More importantly also determined from review of the charts was clinician error when deciding on intravitreal injection frequency.

This resulted in a mixture of a TER and PRN regimen being followed in many patients at IALCH. The results are a more realistic real world experience which has shown that our treatment is effective in treating nAMD however following a TER is not difficult and should easily be adhered to with possible better visual acuity results, fewer clinic visits and fewer injections of intravitreal anti-VEGF.

Areas identified where intervention would make a difference include education of treating ophthalmologists, especially the junior registrars and medical officers, on the TER protocol: Educating patients on the importance of follow-up on correct clinic appointments and the need for close monitoring of patients' response to treatment. The possibility of down referring patients back to base ophthalmology clinics with the adequate staff and equipment for management of nAMD patients. This would be more convenient for the patients as IALCH ophthalmology clinic is only one of two clinics in KwaZulu-Natal currently providing intravitreal

injection of bevacizumab for nAMD and many patients travel a great distance for their clinic appointments.

The results from our study show our VA gain being better than most other studies. The reason is that we did not use the ETDRS charts and ETDRS protocol as most prospective studies did. The VA in our study was recorded by the nursing staff using standard Snellen charts by different nursing staff at each visit in a relatively uncontrolled environment.

In a study comparing VA determined by an ETDRS chart versus a Snellen chart the visual acuity scores were significantly better with the ETDRS charts when compared to Snellen charts. The greatest difference was with poor visual acuity and in patients with nAMD. [5]

Potentially the ETDRS patients in the prospective studies had better initial mean VA and therefore had less of an improvement or gain in letters as seen in our study.

Conclusion

This retrospective chart review has shown that our current treatment for nAMD is resulting in significant visual gains comparable to major prospective trials.

Interim data form the first head-to-head study comparing a TER to the gold standard of a FMR, CANTREAT, has shown very promising results showing a TER is at least effective as a FMR.

At IALCH implementation of a TER protocol was done early because of paucity of resources and personnel and this study has shown that IALCH in not delivering sub-standard care for patients with nAMD but our implementation of the TER can be followed more strictly.

This was a small study but VA gains are similar to that with a FMR with fewer injections and fewer follow-up visits, adding to the growing body of evidence in favour of a TER for the treatment of nAMD.

Study Outcomes	**Fixed Monthly Dosing Studies**				
	ANCHOR [10]	MARINA [10]	CATT-RBZ [15][16]	CATT-BVZ [15][16]	CANTREAT [3]
Mean VA_1y	11.3	7.2	8.5	8.0	6.8
Mean VA_2y	10.7	6.6	8.8	7.8	-
Number Inj_1y	13	13	-	-	11.9
Number Inj_2y	12	12	-	-	
CRT_1y	-	-	196	-	-
CRT_2y	-	-	-	-	-

Table 8: Fixed Monthly Dosing Studies

A TER not only benefits the health sector but the patient as well, saving both money and time.

With fewer injections we can also potentially decrease the risk of complications such as endophthalmitis, cataract, progression of geographic atrophy and arterial thrombotic events from intravitreal injection of anti-VEGF.

Study Outcomes	**PRN Dosing With Monthly Follow-up Studies**			
	CATT-RBZ [15][16]	CATT-BVZ [15][16]	PRONTO [10][14]	Meta-Analysis [7]
Mean VA_1y	6.8	5.9	9.3	5.4
Mean VA_2y	6.7	5.0	11.1	-
Number Inj_1y	-	-	5.6	5.6
Number Inj_2y	-	-	-	-
CRT_1y	-	-	178	100.3
CRT_2y	-	-	-	-

Table 9: PRN Dosing With Monthly Follow-up Studies

BVZ – Bevacizumab. RBZ – Ranibizumab.

Study Outcomes	**TER Studies**			
	Meta-Analysis [7]	RETINA [11]	CANTREAT [3]	**IALCH**
Mean VA_1y	10.4	9.5	7.2	**13**
Mean VA_2y	-	8.0	-	**17**
Number Inj_1y	8.1	8.6	9.9	**9.64**
Number Inj_2y		5.6	-	**6**
CRT_1y	87.7	-	-	**68.1**
CRT_2y	-	-	-	**87.7**

Table 10: TER Studies

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Aldine Oosthuyzen - Manager Information Technology, NWU

Prof. Colleen Aldous – Medical Research Scientist - UKZN

Authors' contributions

Dr E.P. Le Roux was the project leader. Dr E.P. Le Roux and Dr C-H Kruse were responsible for the project design. Calculations were performed by Ms. Aldine Oosthuysen.

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Appendix 1: The final Study Protocol

Appendix 2: The Guidelines for Authorship for the submission Journal

Include the author guidelines in order that the examiner can assess adherence to the journal requirements.

Appendix 3: Ethical approvals

Included hospital and provincial approvals as well as the BREC approval (or waiver if appropriate).

Appendix 4: Raw data

The Administrator, Biomedical Research Ethics Committee, Govan Mbeki Building, University Road, Westville Campus, Tel: 031-260 4769 / 2486 Email: BREC@ukzn.ac.za

Bevacizumab in a treat-and-extend regimen for neovascular age related macular degeneration in a resource limited sector.

DR EP LE ROUX (MBChB)

Ophthalmology Registrar

University of KwaZulu-Natal

PROTOCOL NUMBER:

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BIOMEDICAL RESEARCH ETHICS COMMITTEE
EXPEDITED APPLICATION FORM[1]

Application to the UKZN Research Ethics Committee for ethics review of new research projects
(For research on human participants)

RESEARCH OFFICE CONTACT DETAILS: Biomedical Research Ethics Administration, Westville Campus, Govan Mbeki Building, Private Bag X 54001, Durban, 4000, KwaZulu-Natal, South Africa; Tel: +27 31 2602486; Fax: +27 31 2604609; Email: BREC@ukzn.ac.za ; Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

SECTION A:

APPLICANT/PRINCIPAL INVESTIGATOR:

Title:	Mr		Ms		Mrs		Dr	**DR**	Prof		*(Select option)*	** For UKZN statistical reporting purposes*

Name : **Etienne Philip Le Roux**

*Gender: **Male**

*Race: **White**

UKZN College:

UKZN School/Discipline:	**School of Medicine - Ophthalmology**		
Hospital/Institution where employed:	**King Edward**	NA	
Professional status: **Registrar**		NA	

Postal address: **405 Umhlanga Rocks, Durban, 4320**

Contact phone Numbers: Office: **0313272000**

Mobile number: **0828550907**

Fax number: **0315620904**

Email address:

Full/Part time Employment: **Full Time**

Current HPCSA Number (or equivalent): **MP0609587**
*if registration is pending, submit proof of application

Purpose of research: If postgraduate degree *(Please tick)*	Hons	MMedSc	**MMed**	MSc	MFamMed	MChB	PhD	N/A

Other degree not listed above:

Student Number and year of study: *(if applicable)* **213574210**

If for postgraduate degree, please confirm whether the application has been reviewed and approved by your school's Academic Leader (Research):	**Yes**		No

If yes, provide approval date and attach approval letter:
Will be provided by Prof Adhikari

Name and qualifications of Supervisor
Dr Carl-Heinz Kruse MBCHB, FC Ophthalmology

Name and qualifications of Co-supervisor

[1] Note: This application must be self-sufficient. Sections marked "see protocol" are unacceptable and will be returned to the applicant.

If not for degree purposes, state other (example, self-initiated research):						
Has this study been, or is it likely to be, submitted to any other Research Ethics Committee?	Yes		**No**		N/A	
If yes, please name the Committee/s and or institution and give outcome - i.e. approved/rejected/pending/not applicable? *(If approved, attach approval letter)*						
Please state number of Co-investigators in project:[2] **(if additional space is required for more investigators details please add to the end of application)**						
CO-INVESTIGATOR/S ROLE IN PROJECT ** For UKZN statistical reporting purposes*						
Name:						
Faculty:						
Department:						
*Gender:						
*Race:						
Role:						
Signature of Co-Investigator:						
Name:						
Faculty:						
Department:						
*Gender:						
*Race:						
Role:						
Signature of Co-Investigator:						
Name:						
Faculty:						
Department:						
*Gender:						
*Race:						
Role:						
Signature of Co-Investigator:						
Has the Principal Investigator or any of the co-investigators been previously/or are presently being investigated for alleged research misconduct? *(If yes, please provide details and dates)*	Yes		**No**			
FUNDING OF THE RESEARCH:						
Has funding been secured?	Yes		**No**			

[2] Please note that because of conflict of roles and interests that can arise, academic supervisors and co-investigators should be separate individuals.

Amount: R				
Name of funder: *(full details)*				
Is this project funded from a US DHHS funding source?	Yes		**No**	
If yes, name the federal funding agagency:				
Can this project proceed without funding? *(give a brief explanation)*	**Yes**		No	
Has an application for funds been made to other sources to support this project?	Yes		**No**	
If yes, state name/s of funding agency and amount requested:				

Note:

For all US Federally funded studies (e.g. NIH, CDC, NIAID, DAIDS, NIMH, etc), one complete copy of the original funding application and approval must accompany the BREC ethics application.

All University contracts need to be uploaded on the Contracts Management online submission form with either the signed **Approval letter** (non-research) **or Form 1**(research related). The website link to the system is http://legalservices.ukzn.ac.za/ContractsManagement.aspx

If you require assistance with the completion of the online submission form, or with any aspect of the new system, please contact Mr Rendra Phalad on Ext 7455 for all contracts (non-research contracts), and Mr Deon Moodley on Ext 8199 (for research contracts).

FAILURE TO MAKE FULL FINANCIAL DISCLOSURES WILL DELAY ETHICS APPROVAL

Please indicate whether a BREC review fee is applicable for this study? (See Fee Schedule on BREC Website)	Yes		**No**	
If Yes, is the study covered by your Centre/Unit's annual levy fee to BREC?	Yes		No	

Note:

* Expedited review only applies to minimal risk studies – e.g. retrospective chart reviews, studies on stored samples etc., for details see BREC ToR and SoP at *http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx*

SECTION B:

NATURE OF STUDY

Quantitative

Type of Study: *(please tick)*	Epidemiological	Observational clinical study	Experimental	Observational	
	Retrospective Chart Review	Prospective Chart Review	Laboratory study on stored samples	Audit	Other:(Specify)

Qualitative

1. THE PROTOCOL FOR STUDY

1.1 Full title of research project: *(Please DO NOT use abbreviations or acronyms)*

Bevacizumab in a treat-and-extend regimen for neovascular age related macular degeneration in a resource limited sector.

1.2 Where will the Research be carried out? (Hospital, clinic etc.). **IALCH Eye Clinic**

1.3 Aims (what you hope to achieve) and objectives (how you will achieve your aims) of study: *(please list)*

Aim of study

To obtain the clinical outcomes of patients using intravitreal Bevacizumab with a treat-and-extend regimen for neovascular age-related macular degeneration (AMD) and determine if this regime will benefit resource limited sectors in managing these patients.

Specific objectives

With a monthly regimen of intravitreal Bevacizumab for treatment of patients with neovascular age-related macular degeneration (AMD), the patient burden, especially in the resource limited public setting can be overwhelming to the health system. By using a treat-and-extend regimen we want to achieve the same clinical outcomes for the patients but reduce the number of patient visits to the clinic and reduce the number of intravitreal Bevacizumab injections.

Main objectives

- Mean change from baseline visual acuity.

Other objectives

- Mean change from baseline central macular thickness.
- Mean number of intravitreal Bevacizumab injections.
- Mean maximum period of extension between intravitreal Bevacizumab injections.

1.4 Hypothesis to be tested, or Research Question to be answered:

A treat-and-extend regime has been shown in other clinical trials to be as effective as the gold standard of monthly intravitreal injection of Bevacizumab and may be superior to p.r.n. regime. Treat-and-extend reduces the number of office visits as well as the number of intravireal injections of Bevacizumab and it is important to investigate the visual outcomes and economic impact of this treatment strategy for neovascular AMD in our resource limited setting.

1.5 Summary of the proposed research (restrict to 100 words)

Specific objectives
With a monthly regimen of intravitreal Bevacizumab for treatment of patients with neovascular age-related macular degeneration (AMD), the patient burden, especially in the resource limited public setting can be overwhelming to the health system. By using a treat-and-extend regimen we want to achieve the same clinical outcomes for the patients but reduce the number of patient visits to the clinic and reduce the number of intravitreal Bevacizumab injections.

1.6 Keywords (for database):

Bevacizumab, Treat-and-extend, Neovascular age related macular degeneration, visual acuity, central macular thickness.

1.7 Background and Literature Review (maximum 1 page):

Introduction

Age-related macular degeneration (AMD) also known as age-related maculopathy (ARM) is the leading cause of visual loss in people over 50 years of age in developed countries [1]. AMD is a degenerative disorder affecting the macula and is characterized by the presence of specific clinical findings.
Conventionally AMD is divided into two main types, dry (non-exudative) AMD and wet (exudative/neovascular) AMD. [2] Dry AMD is the most common form with geographic atrophy (GA) a sign of advanced stage of disease. Neovascuar AMD is associated with more rapid progression to advanced sight loss. The main manifestations are choroidal neovascularization (CNV). Untreated CNV forms scar tissue that eventually replaces normal retina tissue, causing irreversible visual loss.

Treatment

The advent of pharmacologic agents that specifically target angiogenesis, such as anti-VEGF, has revolutionized the treatment of neovascular AMD [3]. VEGF increases the growth of abnormal blood vessels and augments vascular permeability. VEGF is up-regulated by hypoxia, and its levels are increased in both the vitreous and retina of patients with retinal vascular disease.

Ranibizumab (Lucentis) is a recombinant anti-VEGF monoclonal antibody fragment that is capable of inhibiting all active forms of VEGF in the eye. Two major studies have examined the effects of ranibizumab in eyes with neovascular AMD. The phase III Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular Age-Related Macular Degeneration (MARINA)Study [4] and The Anti-VEGF Antibody for the Treatment of Predominantly. Classic Choroidal Neovascularization in Age-Related Macular Degeneration (ANCHOR) trial [4] was a 2-year, multicenter, double blind, randomized control trial. MARINA and ANCHOR trials demonstrated that visual acuity outcomes in treating neovascular AMD with fixed monthly intravitreal ranibizumab was far superior to any other treatment to date. [4]

The PRONTO study was the first prospective trial evaluating the use of ranibizumab in a pro re nata (prn) approach to neovascular AMD. The visual outcomes where similar to the to the phase III trials using less than half the injections over the first year. [4] CATT also showed at 1 year that ranimizumab given as needed was not inferior to ranimizumab given monthly. [4]

Bevacizumab (Avastin), is an anti-VEGF monoclonal complete antibody and is very much cheaper than ranibizumab. It is FDA-approved for treatment of metastatic colon cancer. The agent has been used off-label as a treatment option for neovascular AMD having been shown to be relatively safe and effective in several retrospective and prospective studies. [1] The Comparison of AMD Treatment Trials (CATT), a randomized, prospective, head-to-head, non-inferiority trial comparing ranibizumab to bevacizumab has shown that both anti-VEGF agents have equivalent effects on visual acuity when administered according to the same regime. [3]

Treat-and-extend is an alternative type of prn regime that entails administering three initial injections at monthly intervals and then continue monthly injections until the retina is dry. Gradually increasing the period between injections by 2 weeks and continue injections at every visit regardless if retina dry. Continue to extend interval, 12 weeks usually the maximum interval. Able to cut down office visits from 25% to 50% compared to monthly regimen. In a study by Oubraham et al [5], Treat and extend regime had better gain in letters compared to PRN regimen with a few more injections of 8 vs 5 in the PRN group. Two studies by Gupta et al and Shienbaum et al [3 and 4] employed a treat and extend regime of ranibizumab and bevacizumab respectively for treatment of neovascular AMD with favourable visual acuity results with significantly fewer visits and intravitreal injections compared with treatment in a fixed monthly fashion.
While clinical trials are ongoing with novel therapies to decrease the number of office visits and intravitreal injections a treat and extend regime has been shown in other clinical trials to be as effective as the gold standard of monthly intravitreal injection and may be superior to prn regime. Treat and extend reduces the number of office visits as well as the number of intravireal injections and bevacizumab off label is far cheaper than ranibizumab and it is essential to investigate the visual outcomes and economic impact of this treatment strategy for neovascular AMD in our resource limited setting. [3]

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1.8 Key References:
(Give approximately 5 key references)

- 1- Do D, Fallano K, Adyanthaya R et al. Neovascular Age-Related Macular Degeneration. Focal Points. 2010; Module 12: 1-14.
- 2- Kanski J, Bowling B. Clinical Ophthalmology: A Systemic Approach. 2011; 7th Edition:611-627.
- 3- Shienbaum G, Gupta O, Fecarotta C et al. Bevacizumab for Neovascular Age Related Macular Degeneration Using a Treat and Extend Regimen: Clinical and economic Impact. Ophthalmology 2012; 153:468-473.
- 4- Gupta O, Shienbaum G, Patel A et al. A Treat and Extend Regimen using Ranibizumab for Neovascular Age-Related Macular Degeneration. Ophthalmology. 2010; 117:2134-2140.
- 5- Zarbin M, Lim J. Macular Degeneration/Macular Oedema. Audio Digest Ophthalmology. 2014; 52(08):1-4.

2. PLAN OF INVESTIGATION FOR STUDY

* In the case of Higher Degrees, please state name and School of person consulted regarding the design:

2.1	Is this a retrospective chart review with no human contact?	**Yes**		No	
2.2	Is this a study of stored tissue?	Yes		**No**	
2.3	Are host genetic factors being studied?	Yes		**No**	

2.4 How many hours per week will the PI devote to this project? **5 Hours per week**
(Timetable the project in terms of the resources and time available)

3. STATISTICAL PLANNING AND DATA ANALYSIS

3.1	Has this project been approved by a professional statistician? If No, please justify.	**Yes**		No	

3.2 If answered "yes" to (3.1), provide the name of the statistician: Meeting arranged with

Prof Benn Sartorius, Department Public Health, UKZN

3.3 Please provide a brief overview of statistical and data analytic considerations, including:
How was the number of participants determined? Please include assumptions made in any power analysis (e.g. control incidence or mean and standard deviation of primary outcome variable, desired or anticipated effect of treatment or intervention, level of statistical significance and desired power), and list all planned statistical methods to be used. For descriptive studies list statistical operations to be performed.

Sample size

At present around 50 patients will have completed a 2 year follow-up at the end of the study period.

A priori power estimate: Based on a paired t-test assuming a pre-post-test for change in the mean score, a sample size of 50 subjects, assuming a two-tailed alpha level of 0.05 and power of 80% ($\beta = 0.2$) will be able to detect a moderate effect size or a change in visual acuity score /corneal thickness of 0.3 standard deviation units. A Cohen's effect size (or d) [Cohen, 1998] of ≤0.5 is generally considered a moderate effect size.

Cohen, J. (1988). Statistical power analysis for the behavioral sciences (2nd ed.). Hillsdale, NJ: Lawrence Earlbaum Associates.

Data analysis plan

Data will be analysed using Stata 13.0. Continuous variables will be summarized using mean, standard deviation and range (minimum–maximum). If data are skewed then medians and interquartile ranges will be presented. Box plots will also be employed to graphically summarize continuous variables. Categorical data will be represented using frequency tables. Visual acuity will be converted to a logarithm of the minimal angle of resolution score for statistical analysis. A paired T-tests will be used to identify significant change in visual acuity score and central macular thickness based on various pairwise comparisons follow up times points compared to baseline. If these variables are not normal then the non-parametric equivalent of the paired t-test, namely the Wilcoxon test for paired samples, will be used instead. Repeated measures ANOVA may also be employed if significant differences in visual acuity score and central macular thickness comparing baseline to multiple follow up time points. If this data are again not normal then the non-parametric equivalent of the Repeated measures ANOVA, namely the Friedman test, will be employed instead. Correlation between time between injections and impact on visual acuity and central macular thickness change will be assessed using correlation coefficient for paired samples. A p-value of less than 0.05 will be deemed statistically significant in the inferential analyses. Survival analysis will also be employed, namely Kaplan Meier curves, to show the rate of improvement (based on a dichotomous classification of visual acuity from baseline to normal) over the 2-year period.

3.4 For *qualitative* studies: What is the framework/approach to be used for analysis of the data?

4. PARTICIPANTS IN THE STUDY

4.1 Is this a multi-national study? *(If yes, state collaborating countries)*	Yes			**No**		

4.2 List all sites in South Africa in which the project will be carried i.e. geographic location (e.g. KwaZulu-Natal) and type of place (e.g. hospital, clinic, schools, community etc).

KwaZulu Natal, Inkosi Albert Luthuli Academic Hospital, Ophthalmology Clinic

4.3 Source: *(Please indicate number per group)*	Inpatients	**Outpatients** **+/- 50**	Volunteers

4.4 Age (human studies) *(Please indicate number per group)*	Neonates (<28 days)	Infants (1-11 month)	Children (1-12 years)	Adolescent (13-17 years)	**Adults** **All**

4.5 Is there a control group(s)?	Yes		**No**		

4.6 Demographic profile of participants *(please tick ALL appropriate boxes below.)*

4.6.1 Gender: Female [v] Male [v]

4.6.2 Population Group: Black [v] Coloured [v] Indian [v] White [v]

4.6.3 Language Group/s: Specify...English, Afrikaanse, Zulu……

Most patient are White elderly patients with roughly even distribution between male and female.

4.7 Describe the recruitment process in detail for all groups.

All patients that present to IALCH with neovascular AMD in a fixed time period. All patients with suspected neovascular AMD have a fundus fluorescein angiogram to confirm diagnosis.

4.8 Will incentives be offered to facilitate recruitment? *(If yes, describe in detail)*	Yes		**No**		N/A	
4.9 Will participants be reimbursed in some way for participation? *(If yes, describe in detail) See SA DoH Guidelines on BREC Website*	Yes		No		N/A	
4.10 Will reimbursement for participants and investigators be in accordance with: *(If no, please explain)* • Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa: Department of Health (2006) and; • Ethics in Health Research: Principles, Structures and Processes: (2004)? • Current SA DoH Guidance on reimbursement (*See BREC website*)	Yes		No		**N/A**	
4.11 Will participants be insured against research related injury? *(If yes, please provide details; If no, please provide rationale) Mandatory for Clinical Trials*	Yes		No		**N/A**	

4.12 List in detail the inclusion and exclusion criteria.

Inclusion criteria include: Treatment naïve subfoveal choroidal neovascular membrane (CNV) due to neovascular AMD that where treated with a treat-and-extend regime using intravitreal bevacizumab and followed-up for a minimum of 6 months.
Exclusion criteria include: Any previous treatment for neovascular AMD or any prior pars plana vitrectomy surgery.

5. POTENTIAL RISKS OR DISCOMFORT

5.1 Can the project have any potential risks or discomfort on participants, members of the public, researchers, field staff or the physical environment?	Yes		**No**			

5.2 If "yes" to (6.1) indicate, for each study group/arm, the potential additional risks as follows:
5.2.1 Biological risks
5.2.2 Psychological risks
5.2.3 Social Risks
5.2.4 Legal risks
5.2.5 Financial risks
5.2.6 Other risks

5.3 Please detail steps that will be taken to minimise the risks indicated above:
5.3.1 Biological risks
5.3.2 Psychological risks
5.3.3 Social Risks
5.3.4 Legal risks
5.3.5 Financial risks
5.3.6 Other risks

6. INFORMED CONSENT: GIVEN TO PARTICIPANTS

See SAMPLE INFORMATION SHEET AND CONSENT FORM ON UKZN BREC WEBSITE at
http://research.ukzn.ac.za/Libraries/Notices2011/BREC_Informed_consent_form_sflb.sflb.ashx

Other consent forms are acceptable provided that they contain at least the essential elements outlined in the current UKZN BREC Terms of Reference (ToR) and Standard Operating Procedures (SoP) available at
http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

If necessary, information sheets and consent forms, after ethics approval of the English version, must be translated into appropriate local languages and submitted to BREC for further approval prior to implementation, with a copy of the translator's certificate, and back translations if applicable.

The correct and complete contact details for the UKZN Biomedical Research Ethics Committee should be in the information sheets and consent forms as follows:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus
Govan Mbeki Building
University of KwaZulu-Natal
Private Bag X 54001, Durban, 4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2602486 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

7. DECLARATION OF PRINCIPAL INVESTIGATOR

Conflict of Interest:

I declare that all potential conflicts of interest regarding my application for ethics approval to conduct this study have been declared in accordance with UKZN and BREC Terms of Reference and Standard Operating Procedures.

Undertaking:

I understand and accept that I will be required to submit a yearly recertification application, failing which authorisation to continue the study lapses.
I undertake to request permission for any changes/amendments to the study from BREC in advance of implementing any such changes, unless they are emergencies required to prevent harm or save life. In such cases BREC must be notified urgently.

I agree to provide monitoring data if and when required.

I expect the project to be completed by **DATE**...**08/12/2015**.....

I agree to abide by the guidance contained in the SA Department of Health (2004) Ethics in Health Research: Principles, structures and processes and the (2006) South African Good Clinical Practice Guidelines and the current UKZN Biomedical Research Ethics Committee Terms of Reference and Standard Operating Procedures. These are available at *http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx*

I understand and accept that all information pertaining to this application is a true reflection of the project proposed and I take full responsibility should there be any transgression.

SIGNATURE OF PRINCIPAL INVESTIGATOR.....

FULL NAME OF PRINCIPAL INVESTIGATOR... ETIENNE P. LE ROUX

DATE.....05/11/2014.....

8. DECLARATION AND APPROVAL FROM SUPERVISOR AND CO-SUPERVISOR (if applicable)

(I HAVE READ AND CHECKED THE PROPOSAL AND IT IS READY FOR SUBMISSION;

Remarks:

SIGNATURE OF SUPERVISOR .....

FULL NAME OF SUPERVISOR.....Dr C. KRUSE.....

DATE.....05/11/2014.....

SIGNATURE OF CO-SUPERVISOR .....

FULL NAME OF CO-SUPERVISOR.....

DATE.....

If applicable, attach a signed copy of the Supervision Agreement between the student, supervisor and any co-supervisor.

9. DECLARATION AND APPROVAL OF LINE MANAGER
(Must include verification of interdepartmental agreements and co-operation)

Remarks:

SIGNATURE OF LINE MANAGER .....

FULL NAME OF LINE MANAGER L. VISSER (HoD)

DATE 12/11/2014

NB: If applicant is ACADEMIC LEADER/DEAN/HOS, the ACADEMIC LEADER'S/DEAN'S/HOS's Line Manager (DVC) must sign.

SIGNATURE OF ACADEMIC LEADER's/ HOS's/DEAN's Line Manager.....

FULL NAME OF ACADEMIC LEADER's, HOS's/DEAN's Line Manager.....

DATE.....

SUGGESTED CURRICULUM VITAE FORMAT

(3 COPIES MAXIMUM 4 PAGES)

CURRICULUM VITAE (of Principal Investigator and all Co-Investigators)
(CVs to be completed and signed for each member of the research team)

Full name:
Date of birth:
Male/Female:
Telephone (Home):
Telephone (Business):
Cell:
Fax No:
E-mail Address:
Current HPCSA No: **(or equivalent statutory health council registration No. as appropriate)**
Present position:
Institution:
Department/Section:
Nationality/Permanent residency:
Previous positions held (last 10 years):
Qualifications:
University where obtained/year:
Area of study:
Number of Postgraduate theses supervised (Masters and Doctoral):
Publication list over the past 3 years:
Details of all other research studies presently being conducted:
Certificate of recent (past 3 years) research ethics and/or GCP training (GCP required for clinical trials):

CHECKLIST FOR BIOMEDICAL RESEARCH ETHICS APPLICATIONS

NB: DO NOT BIND SUBMISSIONS (STAPLE ONLY)

Applications to be addressed to: The Administrator, Biomedical Research Ethics Committee, Govan Mbeki Building, University Road, Westville Campus, Tel: 031-260 4769 / 2486 Email: BREC@ukzn.ac.za

Note to Students:

PLEASE NOTE THAT ONLY ONE COPY OF APPLICATION AND SUPPORTING DOCUMENTS NEED BE SUBMITTED IF STUDY IS FOR DEGREE PURPOSES. ALL APPLICATIONS FOR DEGREE PURPOSES MUST BE SUBMITTED VIA THE COLLEGE POST-GRADUATE OFFICE WITH AN APPROVAL LETTER ATTACHED.

IF STUDY IS FOR NON-DEGREE PURPOSES THEN 3 COPIES MUST BE SUBMITTED TO BREC.

INCOMPLETE SUBMISSIONS MAY RESULT IN DELAYED REVIEW OF THE APPLICATION

For all expedited review applications:

- **3 TYPEWRITTEN COPIES** OF APPLICATION (Back-to-back (double-sided) copies preferred)
- **3 COPIES** OF THE PROTOCOL
- **3 COPIES** OF CURRENT CV/s (abbreviated max 4 PAGES)
- **3 COPIES** OF EVIDENCE OF CURRENT GCP / RESEARCH ETHICS TRAINING
- **3 COPIES** OF ALL QUESTIONNAIRES TO BE USED IN THE STUDY
- **3 COPIES** OF THE INFORMED CONSENT FORMS (See BREC templates)
- **3 COPIES** OF THE PATIENT INFORMATION LEAFLET (See BREC templates)
- HAVE YOU FAMILIARISED YOURSELF WITH THE BREC TERMS OF REFERENCE? (See http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx)
- DETAILS OF ALL FUNDING SUPPORT?
- ALL PERSONAL INFORMATION?
- ANSWERED ALL QUESTIONS?
- GIVEN DETAILS OF ALL RESEARCH PRESENTLY BEING UNDERTAKEN?
- DELETED UNNECESSARY BLANK SPACES IN THE DOCUMENT?
- **IS DECLARATION PAGE SIGNED BY PI/SUPERVISOR AND ACADEMIC LEADER/HOS/DEAN/LINE MANAGER?**

Protocol

Bevacizumab in a treat-and-extend regimen for neovascular age related macular degeneration in a resource limited sector.

DR EP LE ROUX (MBChB)

Ophthalmology Registrar

University of KwaZulu-Natal

Title of study

Bevacizumab in a treat-and-extend regimen for neovascular age related macular degeneration in a resource limited sector.

Aim of study

To obtain the clinical outcomes of patients using intravitreal Bevacizumab with a treat-and-extend regimen for neovascular age-related macular degeneration (AMD) and determine if this regime will benefit resource limited sectors in managing these patients.

Specific objectives

With a monthly regimen of intravitreal Bevacizumab for treatment of patients with neovascular age-related macular degeneration (AMD), the patient burden, especially in the resource limited public setting can be overwhelming to the health system. By using a treat-and-extend regimen we want to achieve the same clinical outcomes for the patients but reduce the number of patient visits to the clinic and reduce the number of intravitreal Bevacizumab injections.

Main objectives

- Mean change from baseline visual acuity.

Other objectives

- Mean change from baseline central macular thickness.
- Mean number of intravitreal Bevacizumab injections.
- Mean maximum period of extension between intravitreal Bevacizumab injections.

Background and Literature

Introduction

Age-related macular degeneration (AMD) also known as age-related maculopathy (ARM) is the leading cause of visual loss in people over 50 years of age in developed countries [1]. AMD is a degenerative disorder affecting the macula and is characterized by the presence of specific clinical findings.

Conventionally AMD is divided into two main types, dry (non-exudative) AMD and wet (exudative/neovascular) AMD. [2] Dry AMD is the most common form with geographic atrophy (GA) a sign of advanced stage of disease. Neovascuar AMD is associated with more rapid progression to advanced sight loss. The main manifestations are choroidal neovascularization (CNV). Untreated CNV forms scar tissue that eventually replaces normal retina tissue, causing irreversible visual loss.

The aetiology of macular degeneration is not fully understood. Genetic risk factors and environmental risk factors, such as smoking, may contribute to the development and progression of AMD. Smoking roughly doubles the risk of AMD.[1] Age is another major risk factor. Late ARM is more common in Caucasians than other races, despite a similar prevalence of early ARM.

Hypertension, high fat intake and obesity, cataract surgery, blue iris colour, high sunlight exposure, and female gender are suspected. [2]

Micronutrient supplementation is recommended in eyes with intermediate or advanced AMD. When individuals progress to advanced AMD with CNV under the fovea, treatment with **intravitreal vascular endothelial growth factor (VEGF) inhibitors is the current standard of care.** [1]

Treatment

The first effective treatment of CNV was thermal laser photocoagulation. In 1982 the **Macular Photocoagulation Study Group** [1] reported that patients receiving argon laser treatment to extrafoveal lesions had a decreased rate of visual decline compared to untreated control group. Evidence that laser photocoagulation was better than observation alone for juxtafoveal and subfoveal CNV was also observed. This treatment however caused irreversible damage to the overlying neurosensory retina, as well as a recurrence rate of more than 50% of CNV.

Photodynamic therapy (PDT) in the late 1990s offered an alternative to thermal laser photocoagulation. PDT selectively targets CNV without damaging the overlying retinal tissue. Verteporfin, an intravenously injected photosensitizing dye, preferentially accumulates within the neovascular tissue. Non-thermal laser light activation then follows, leading to free radical formation, endothelial damage, and thrombosis of the choroidal vessels. **The Therapy of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Study Group** [1] conducted a randomized trial comparing PDT with placebo and analyzed visual acuity loss between the 2 groups. At 2 years of follow-up after randomization, 53% of PDT patients lost 15 or fewer letters of visual acuity, compared to 38% of placebo patients which was statistically significant. PDT was however less effective in some CNV types. PDT is less destructive method of treatment compared to thermal photocoagulation but was not beneficial in all CNV types and was not associated with visual acuity gain.

The advent of pharmacologic agents that specifically target angiogenesis, such as **anti-VEGF, has revolutionized the treatment of neovascular AMD**.[3] VEGF increases the growth of abnormal blood vessels and augments vascular permeability. VEGF is up-regulated by hypoxia, and its levels are increased in both the vitreous and retina of patients with retinal vascular disease.

Pegaptanib sodium (Macugen) was the first drug directed against VEGF. Pegaptanib is an aptamer, a small molecule of RNA attached to polyethylene glycol that directly binds to $VEGF_{165}$ and prevents it from activating DNA transcription. Pegaptanib is administered by intravitreal injection every six weeks. While the initial results in the **VISION trials** [1] were promising, the majority of patients in the pegaptanib clinical trials still lost vision, and the treatment is considered one that slows the disease rather than one that improves visual acuity.

Ranibizumab (Lucentis) is a recombinant anti-VEGF monoclonal antibody fragment that is capable of inhibiting all active forms of VEGF in the eye. Two major studies have examined the effects of ranibizumab in eyes with neovascular AMD. The phase III Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular Age-Related Macular Degeneration **(MARINA)Study** [4] and The Anti-VEGF Antibody for the Treatment of Predominantly. Classic Choroidal Neovascularization in Age-Related Macular Degeneration **(ANCHOR) trial** [4] was a 2-year,

multicenter, double blind, randomized control trial. **MARINA and ANCHOR trials demonstrated that visual acuity outcomes in treating neovascular AMD with fixed monthly intravitreal ranibizumab was far superior to any other treatment to date**. [4]

Alternative fewer fixed scheduled injections have not resulted in visual outcomes that were as favorable**. PIER trial** [5] showed quarterly dosing of Ranibizumab after 3 monthly doses inferior to monthly dosing. **EXCITE trial** [5] also showed monthly fixed injection to be superior to three monthly injections and then quarterly injections. **HORIZON trial** [5] showed visual acuity decreased when patients from the MARINA and ANCHOR trial switched to prn dosing followed 6 monthly then changed to 3 monthly follow-up. **SAILOR trial** [5] also showed monthly dosing to be superior.

The PRONTO study was the first prospective trial evaluating the use of **ranibizumab in a pro re nata (prn) approach** to neovascular AMD. The visual outcomes where similar to the to the phase III trials using less than half the injections over the first year. [4] **CATT also showed at 1 year that ranimizumab given as needed was not inferior to ranimizumab given monthly.** [4]

Bevacizumab (Avastin), is an anti-VEGF monoclonal complete antibody and is very much cheaper than ranibizumab. It is FDA-approved for treatment of metastatic colon cancer. The agent has been used off-label as a treatment option for neovascular AMD having been shown to be relatively safe and effective in several retrospective and prospective studies. [1] The Comparison of AMD Treatment Trials **(CATT), a randomized, prospective, head-to-head, non-inferiority trial comparing ranibizumab to bevacizumab has shown that both anti-VEGF agents have equivalent effects on visual acuity when administered according to the same regime.** [3]

Aflibercept (Eylea) or VEGF Trap-Eye binds all forms of VEGF-A to D as well as Placental Growth Factor. **VIEW 1 and 2 trials** [5] showed all doses of aflibercept dosing of 2mg q 4wk/ 0.5mg q 4wk/ 2mg q 8wk non-inferior to ranibizumab 0.5mg q 4wk.

Treatment options of bevacizumab:

- **Fixed monthly injection** is the regimen adopted in initial major trials. Overall, around 95% of patients maintain vision regardless of lesion type, and 35–40% significantly improved, most markedly during the first 3 months.

- **Pro re nata (prn) regime** with three initial monthly injections followed by monthly review with re-injection when deterioration occurs as assessed by VA and OCT. Very important is that patients must continue being followed-up monthly as not to miss recurrences. Even with monthly follow-up patients treated prn may have multiple recurrences and may lose vision over time.

- **Treat-and-extend is an alternative type of prn regime** that entails administering three initial injections at monthly intervals and then continue monthly injections until the retina is dry. Gradually increasing the period between injections by 2 weeks and continue injections at every visit regardless if retina dry. Continue to extend interval, 12 weeks usually the maximum interval. Able to cut down office visits from 25% to 50% compared to monthly regimen. In a study by **Oubraham et al** [5], Treat and extend regime had better gain in letters compared to PRN regimen with a few more injections of 8 vs 5 in the PRN group. **Two studies by Gupta et al and Shienbaum et al** [3 and 4] employed a treat and extend regime of ranibizumab and bevacizumab respectively for treatment of neovascular AMD

with favourable visual acuity results with significantly fewer visits and intravitreal injections compared with treatment in a fixed monthly fashion.

While clinical trials are ongoing with novel therapies to decrease the number of office visits and intravitreal injections a treat and extend regime has been shown in other clinical trials to be as effective as the gold standard of monthly intravitreal injection and may be superior to prn regime. Treat and extend reduces the number of office visits as well as the number of intravireal injections and bevacizumab off label is far cheaper than ranibizumab and is essential to investigate the visual outcomes and economic impact of this treatment strategy for neovascular AMD in our resource limited setting. [3]

Key References

- 1- Do D, Fallano K, Adyanthaya R et al. Neovascular Age-Related Macular Degeneration. Focal Points. 2010; Module 12: 1-14.
- 2- Kanski J, Bowling B. Clinical Ophthalmology: A Systemic Approach. 2011; 7th Edition:611-627.
- 3- Shienbaum G, Gupta O, Fecarotta C et al. Bevacizumab for Neovascular Age Related Macular Degeneration Using a Treat and Extend Regimen: Clinical and economic Impact. Ophthalmology 2012; 153:468-473.
- 4- Gupta O, Shienbaum G, Patel A et al. A Treat and Extend Regimen using Ranibizumab for Neovascular Age-Related Macular Degeneration. Ophthalmology. 2010; 117:2134-2140.
- 5- Zarbin M, Lim J. Macular Degeneration/Macular Oedema. Audio Digest Ophthalmology. 2014; 52(08):1-4.

Study design

A retrospective chart review.

A retrospective study uses existing data that have been recorded for reasons other than research.

Study population

All patients with neovascular age related macular degeneration (AMD) that present at Inkosi Albert Luthuli Central Hospital (IALCH) in a fixed time period. AMD is more common in the elderly Caucasian population.

Sampling strategy

All patients that present to IALCH with neovascular AMD in a fixed time period. All patients with suspected neovascular AMD have a fundus fluorescein angiogram to confirm diagnosis.

Sample size

At present around 50 patients will have completed a 2 year follow-up at the end of the study period.

A priori power estimate:

Based on a paired t-test assuming a pre-post-test for change in the mean score, a sample size of 50 subjects, assuming a two-tailed alpha level of 0.05 and power of 80% ($\beta = 0.2$) will be able to detect a moderate effect size or a change in visual acuity score /corneal thickness of 0.3 standard deviation units. A Cohen's effect size (or d) [Cohen, 1998] of ≤ 0.5 is generally considered a moderate effect size.

Cohen, J. (1988). Statistical power analysis for the behavioral sciences (2nd ed.). Hillsdale, NJ: Lawrence Earlbaum Associates.

Inclusion / exclusion criteria

Included are all patients that presented to IALCH with neovascular AMD in a fixed time period and complete a fixed follow-up in that period.

Inclusion criteria include: Treatment naïve subfoveal choroidal neovascular membrane (CNV) due to neovascular AMD that where treated with a treat-and-extend regime using intravitreal bevacizumab and followed-up for a minimum of 6 months.

Exclusion criteria include: Any previous treatment for neovascular AMD or any prior vitrectomy surgery.

Data collection methods and tools

All patients with the diagnosis of neovascular AMD in a fixed time period will have their files analysed. All patient records are already in electronic format at IALCH. I will review the relevant patient charts and enter them onto an Excel spreadsheet. To check data for accuracy I will perform random checks on data entered. All patient records are stored at IALCH in electronic format and will be easy to trace data back to the original patient charts.

The following data will be collected: sex, age, date of neovascular AMD diagnosis, best corrected visual acuity at time of diagnosis and at every visit, analysis of pre-treatment fluorescein angiogram to establish CNV lesion size, OCT analysis and evaluation of central retinal thickness, intraretinal fluid and subretinal fluid at time of diagnosis and at every visit, number of intravitreal Bevacizumab injections over a fixed time period and mean maximum period between injections.

Data analysis plan

Data will be analysed using Stata 13.0. Continuous variables will be summarized using mean, standard deviation and range (minimum–maximum). If data are skewed then medians and interquartile ranges will be presented. Box plots will also be employed to graphically summarize continuous variables. Categorical data will be represented using frequency tables. Visual acuity will be converted to a logarithm of the minimal angle of resolution score for statistical analysis. A paired

T-tests will be used to identify significant change in visual acuity score and central macular thickness based on various pairwise comparisons follow up times points compared to baseline. If these variables are not normal then the non-parametric equivalent of the paired t-test, namely the Wilcoxon test for paired samples, will be used instead. Repeated measures ANOVA may also be employed if significant differences in visual acuity score and central macular thickness comparing baseline to multiple follow up time points. If this data are again not normal then the non-parametric equivalent of the Repeated measures ANOVA, namely the Friedman test, will be employed instead. Correlation between time between injections and impact on visual acuity and central macular thickness change will be assessed using correlation coefficient for paired samples. A p-value of less than 0.05 will be deemed statistically significant in the inferential analyses. Survival analysis will also be employed, namely Kaplan Meier curves, to show the rate of improvement (based on a dichotomous classification of visual acuity from baseline to normal) over the 2-year period.

Study location

IALCH Eye Clinic

Study period

From the initiation of the treat and extend regime at IALCH eye clinic until final data collection is completed in 2015.

Limitations to the study

- Retrospective chart review
- Lack of control group
- Use of Snellen chart and not EDTRS chart for visual acuity
- Relative small sample size

Ethical considerations

No patient names or identifying details will be used in the data collection or publication of the information.

DR EP LE ROUX (MBChB)

Ophthalmology Registrar

University of KwaZulu-Natal

CURRICULUM VITAE - Supervisor

CURRICULUM VITAE (of Principal Investigator and all Co-Investigators)
(CVs to be completed and signed for each member of the research team)

Full name: Dr Carl-Heinz Kruse

Date of birth: 1975-06-29

Male/Female: Male

Telephone (Home): 031 811 8130

Telephone (Business): 033 897 3345

Cell: 084 011 0767

Fax No: 033 897 3111

E-mail Address: ruraleye@gmail.com

Current HPCSA No: MP 0532851

Present position: Head of Clinical Unit

Institution: Grey's Hospital

Department/Section: Ophthalmology

Nationality and Permanent residency: RSA

Previous positions held (last 10 years):

Principal Specialist: Ngwelezana Hospital (2008 - 2010)

Registrar: St Aidan's Hospital (2004 - 2007)

MO: Ermelo Hospital, Edendale Hospital (2002 - 2004)

Qualifications: MBChB: University of Pretoria 2000

MMed(Ophth): UKZN 2008

FCOphth: CMSA 2007

Area of study: Ophthalmology

Number of Postgraduate theses supervised (Masters): 4

Publication list over the past 3 years:

- Kruse C. What is the ideal treatment of diabetic macular oedema? *S Afr J Diabetes Vasc Dis* 2013;**10**:120-121
- Kruse C. The effects of systemic medication on diabetic retinopathy. *S Afr J Diabetes Vasc Dis* 2014;**11**:113-114

Details of all other research studies presently being conducted: -

CURRICULUM VITAE
ETIENNE PHILIP LE ROUX

RESIDENTIAL ADDRESS	79 William Campbell Street La Lucia, Durban 4051
TELEPHONE NUMBERS	South Africa Cell: +27 (0) 828550907
E-MAIL ADDRESS	epleroux@yahoo.com
1. PERSONAL DATA	
SURNAME	Le Roux
FIRST NAMES	Etienne Philip
DATE OF BIRTH	19 October 1980
IDENTITY NUMBER	8010195098083
PASSPORT NUMBER	M00019877 Republic of South Africa
MARITAL STATUS	Married
DEPENDANTS	2 - Male D.O.B 22/02/2011 Male D.O.B. 27/03/2012
LANGUAGE PROFICIENCY	English – Fluent Afrikaans – Fluent
DRIVERS LICENCE	Code B & own transport
HEALTH	Excellent (Non-Smoker)
RELIGION	Christian
2. ACADEMIC QUALIFICATIONS	
EDUCATION	
PRIMARY SCHOOL ATTENDED	Nooitgedacht Primary School Gauteng Province (1987-1993)
SPECIAL ACHIEVEMENTS	Provincial Cross Country colours Provincial Judo colours
HIGH SCHOOL ATTENDED	Hyde Park High School Gauteng Province (1993-1998)
HIGHEST GRADE PASSED	Grade 12 with full exemption
HIGHER EDUCATION	
UNIVERSITY	University of Pretoria Faculty of Health Sciences
DEGREE	MBCHB
SUBJECTS	Complied with all the requirements for the degree MBCHB
COLLEGE OF MEDICINE OF SOUTH AFRICA	
	FC Ophth(SA) Primary 1A – September 2011
	Diploma in Ophthalmology March 2012
	FC Ophth(SA) Intermediate 1(B) - 2013

3. PREVIOUS EMPLOYMENT	
POSITION	**Gauteng Department of Health:** **Leratong Hospital** *Period Employed:* 01/01/2005-31/12/2005 *Job Description:* Intern Medical Officer *Responsibilities:* Internal Medicine - 2 months Obstetrics and Gynecology - 4 months Pediatrics - 2 months Surgery - 4 months Anesthesiology - 2 weeks
POSITION	**Mpumalanga Department of Health:** **Kwamhlanga Hospital** *Period Employed:* 01/01/2006-31/12/2006 *Job Description:* Community Service M.O. *Responsibilities:* Obstetrics and Gynecology - 2 months Pediatrics - 2 months Surgery and Orthopedics - 4 months Casualty and Rural Clinics - 4 months
POSITION	**Republic of Ireland:** **St Lukes Hospital** *Period Employed:* 17/01/2007 – 30/06/2007 *Job Description:* Senior House Officer A&E *Responsibilities:* Treatment of trauma and surgical related emergencies presenting to the accidents and emergencies department.
POSITION	**Gauteng Department of Health:** **Tembisa Hospital** *Period Employed:* 23/07/2007-30/11/2007 *Job Description:* Senior House Officer Surgery *Responsibilities:* Management of surgical patients, daily ward rounds, out patients, casualty, assisting in theater, minor surgical procedures, ICU.
POSITION	**Democratic Republic of the Congo:** **Crusader Health DRC - KCC Mine** *Period Employed:* 01/12/2007-30/09/2009 *Job Description:* General Practitioner *Responsibilities:* Out patients mine employees and their dependants, emergencies and tropical medicine. Occupational health for a copper mine and process plant with open pit and underground mining operations including annual medicals, risk based medical surveillance, statistic reporting and risk assessments.
POSITION	**Zambia:** **Crusader Health Zambia – Frontier Mine** *Period Employed:* 01/10/2009-15/10/2010 *Job Description:* Clinic Administrator *Responsibilities:* The opening and administration of a new

	14 bed mine clinic. Out patients department, child health, anti-natal and maternity, family planning, ARV clinic, maternity, emergency room and occupational health.
POSITION	**Gauteng Department of Health:** **Tshwane District Hospital** *Period Employed:* 15/10/2010-31/05/2011 *Job Description:* **Supernumerary position in Ophthalmology** *Responsibilities:* Ophthalmology clinic, academic rounds
POSITION	**Mpumalanga Department of Health:** **Witbank Hospital** *Period Employed:* 01/06/2011-30/06/2013 *Job Description:* **M.O. Ophthalmology** *Responsibilities:* - Ophthalmology clinic - Ophthalmology Call's - Ward consultations - Academic Rounds at Steve Biko - Theatre
CURRENT POSITION	**KZN Department of Health:** **King Edward Hospital** Period Employed: 01/07/2013-present Job Description: Registrar Ophthalmology Responsibilities: - Ophthalmology clinic - Ophthalmology Call's - Ward consultations - Academic Rounds - Theatre
4. HOBBIES AND LESIURE ACTIVITIES	
	Long distance running, golf, reading, judo
5. ADDITIONAL COURSES	
	Basic Life Support 2006,2008,2010 Advanced Cardiac Life Support 2006,2008,2010 Advanced Trauma Life Support 2006 Advanced Pediatric Life Support 2008 Basic Surgical Skills 2009 Ethics in Trauma 2010 **Micro-Surgical Skills Course 2011** **Alcon Introductory Phaco Course 2011**
5. SYMPOSIUMS	
	Jacaranda Symposium 2011 **SAGS 2012**
6. CATARACT TOURS	**Moses Kotane Hospital October 2012**
6. MEMBERSHIP OF PROFESSIONAL	

BODIES	
	Health Profession Council of South Africa Medical Council of Ireland Medical Protection Society South African Medical Association
7. REFERENCES	
Dr Baltazaar **Consultant General Surgery** **Leratong Hospital**	+27 11 4113500
Prof GA Ogunbanjo **Academic Coordinator Mpumalanga** **Kwamhlanga Hospital**	+27 13 9479100
Mr F. O'Dywer **Consultant Accidents and Emergencies** **St Luke's Hospital**	+353 567785000
Mike Clifford **Director Crusader Health**	+ 27 721359854
Dr Musutwana **Consultant Department Ophthalmology** **Witbank Hospital**	+27 828569270

9910437

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Bachelor of Medicine and Surgery

with all the associated rights and privileges
was conferred on

ETIENNE PHILIP LE ROUX

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SURNAME: **LE ROUX**

FIRST NAMES: **ETIENNE PHILIP**

REGISTRATION NUMBER: **MP 0609587**

QUALIFICATIONS: **MB ChB Pret 2004**

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Tel +27 11 726 7037 Fax +27 11 726 4036
Email: Academic Registrar: alv@cmsa-jhb.co.za
General: admin@cmsa-jhb.co.za
Website: http//www.collegemedsa.ac.za

20 October 2011

Dr EP le Roux
45 Maldon Road
LYNNWOOD GLEN
0081

Dear Doctor

FC Ophth(SA) Primary 1A

I have pleasure in confirming your success in the recent CMSA examination, and in congratulating you on behalf of the President and the Members of Senate of the CMSA.

Yours faithfully

Prof MM Sathekge
HONORARY REGISTRAR

MMS/ALV/elp

The Colleges of Medicine

of

South Africa

We declare

Etienne Philip le Roux

by reason of academic and professional attainments a

Diplomate in Ophthalmology

of the

College of Ophthalmologists of South Africa

President

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Member of Senate

24 May 2012

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SURNAME
LE ROUX

FORENAMES
ETIENNE PHILIP

COUNTRY OF BIRTH
SOUTH AFRICA

DATE OF BIRTH
1980-10-19

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- Ensure that ‘AIDS’ is used for the disease and ‘HIV’ for the virus, e.g. do not use ‘AIDS carrier’, ‘AIDS positive’, ‘AIDS virus’ or ‘catching AIDS or HIV/AIDS’ (avoid using the solidus here).
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  - for ‘man-made fibre’ use ‘synthetic fibre’
- **‘He/she’, ‘him/her’ and ‘his/hers’:** For ‘he/she’, ‘him/her’ and ‘his/hers’ rather use ‘he o**r** she’, ‘her o**r** him’, ‘his or hers’ (without a solidus) or change to plural ‘they’. Use inclusive pronouns: use ‘he or she’, or rephrase the sentence (rephrasing to the plural form often works):

  ✗ … *Any observer* of changes in publishing technology will perceive that *he* has need of…

  ✓ **… *Observers*** of… will perceive that *they* have…

  Beware of referring to people with stereotypical pronouns (e.g. ‘the doctor treated *his* patient’; ‘the secretary tidied *her* desk’).

- **Geography**
  - The terms *Third World*, *poor countries* and *underdeveloped countries* should be avoided.
  - *Developing* or *non-developed country/society* is better, but it is best to specify countries or regions instead.
  - *Western society* and *Western World* should only be used in relation to geography; otherwise, use *developed world/society* or, even better, specify the countries themselves or the region.

Tables, figures and photographs ↥

In Step 4 of the online submission process, upload all tables, figures, images, and supplementary files. Tables should be saved and uploaded as separate Excel (.xls) files with no more than 10 figures and tables in total per article. Ensure that all personal identifying information is removed from the supplementary files as indicated in the provided instructions. All captions should be provided together on a separate page. Tables and figures should use numerical numbers.

- **Organise your visual presentation:** Once you have read through the analyses and decided how best to present each table or figure, think about how you will arrange them within the article. The analyses should tell a story' that leads the reader through the steps needed to logically answer the question(s) that you as author are posing in the Introduction. The order in which you present the results can be as important in convincing the readers as what you actually are saying in the text.
- **How to refer to tables and figures in the text:** Every figure and table included in the paper *must* be referred to in the body of the text. Use sentences that draw the reader's attention to the relationship or trend you wish to highlight, referring to the appropriate figure or table only in parenthesis e.g.:
  - Germination rates were significantly higher after 24 h in running water than in controls (Figure 4).
  - DNA sequence homologies for the purple gene from the four congeners (Table 1) show high similarity, differing by at most 4 base pairs. (Avoid sentences that give no information other than directing the reader to the figure or table, e.g. Table 1 shows the summary results for male and female heights at Bates College.)
- **Abbreviation of the word 'Figure':** When referring to a figure in the text, the word 'figure' is never abbreviated as 'Fig.'; the same rule applies to the usage of 'table'. Both words are spelled out completely in descriptive legends.
- **How to number tables and figures:** Figures and tables are numbered independently, in the sequence in which you refer to them in the text, starting with Figure 1 and Table 1. If, in revision, you change the presentation sequence of the figures and tables, you must renumber them to reflect the new sequence.
- **The acid test for tables and figures:** Any table or figure you present must be clear, well-labelled, and described by its legend to be understood by your intended audience without reading the results section. That is, it must be able to stand alone and be interpretable. Overly complicated figures or tables may be difficult to understand in or out of context, so strive for simplicity whenever possible.
- **Descriptive legends or captions:** To pass the acid test above, a clear and complete legend (sometimes called a caption) is essential. Like the title of the article itself, each legend should convey as much information as possible about what the table or figure intends to tell the reader:
  - the results that are being shown in the graph(s), including the summary statistics plotted
  - the organism studied in the experiment (if applicable)
  - a context for the results: the treatment applied or the relationship displayed, etc.
  - location (*only* if a field experiment)
  - specific explanatory information needed to interpret the results shown (in tables, this is frequently done as footnotes)
  - culture parameters or conditions if applicable (temperature, media, etc.)
  - sample sizes and statistical test summaries, as they apply

  Do not simply restate the axis labels with a 'versus' written in between.

  Example: Figure 1: Height frequency (%) of White Pines (Pinusstrobus) in the Thorncrag Bird Sanctuary, Lewiston, Maine, before and after the Ice Storm of 1998. Before, $n = 137$, after, $n = 133$. Four trees fell during the storm and were excluded from the post-storm survey.

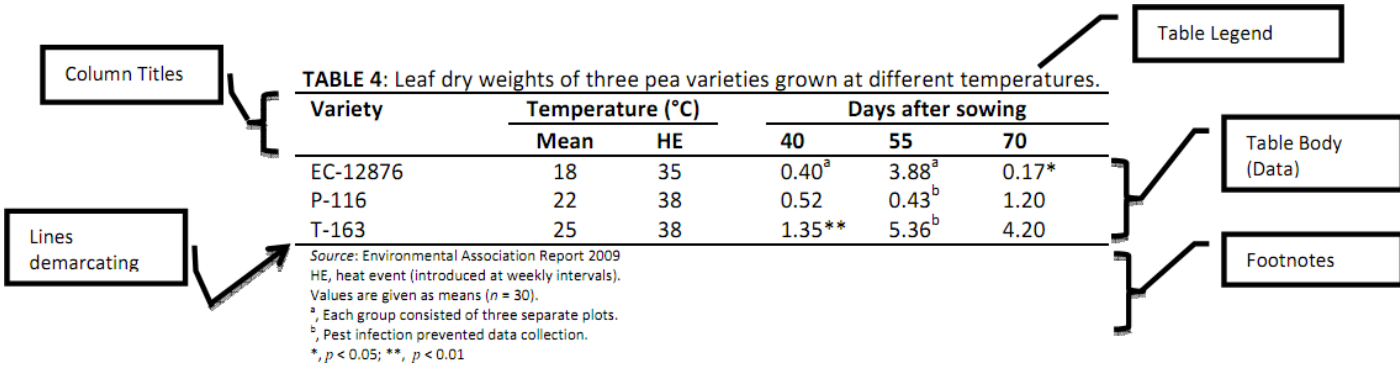

TABLE 4: Leaf dry weights of three pea varieties grown at different temperatures.

Variety	Temperature (°C)		Days after sowing		
	Mean	HE	40	55	70
EC-12876	18	35	0.40[a]	3.88[a]	0.17*
P-116	22	38	0.52	0.43[b]	1.20
T-163	25	38	1.35**	5.36[b]	4.20

Source: Environmental Association Report 2009
HE, heat event (introduced at weekly intervals).
Values are given as means (n = 30).
[a], Each group consisted of three separate plots.
[b], Pest infection prevented data collection.
*, $p < 0.05$; **, $p < 0.01$

Note: Questions frequently arise about how much methodology to include in the legend, and how much results reporting should be done. For laboratory reports, specific results should be reported in the results text with a reference to the applicable table or figure. Other than culture conditions, methods are similarly confined to the Methods section.

Footnotes to tables, figures and photographs

Do not introduce footnotes in the body of the article. Footnotes should be used as follows:

- Copyright and permissions to reproduce should be clearly stated.
- Notes about the table as a whole can be left unlinked (i.e. no linking letters or numbers or symbols) or linked to, for example, a relevant column heading.
- Notes about specific parts of the table should be linked using superscript lower case letters (preferred), superscript numbers or symbols.
- If lower case letters are used, it could be confused with the table data; use symbols or numbers instead.
- Do not make use of superscript numbers in parentheses (brackets).
- If an abbreviation is mentioned for the first time in a table (e.g. 'CE' in Table 1), it must be defined in a footnote to that table, (e.g. HE, Heat event (introduced at weekly intervals).
- Asterisk footnotes are reserved for probability values in tables and usually signify the following values: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$. The asterisk is often used in mathematics and should therefore be avoided as a footnote symbol.
- Footnote links should be placed after punctuation. The preferred order of footnote symbols in tables (which should be superscripted) is †, ‡, §, ¶ (these are doubled if more footnotes are needed, e.g. ††).
- When superscript numbers or letters are used in text, beware of potential confusion with other superscripts (e.g. 2 for 'squared').
- Footnotes should be in the following order:
  - source notes
  - other general notes
  - notes on specific parts of the table (following the order in the table itself)
  - notes on level of probability

Guidance on submitting creatives electronically

Supply your manuscript creatives in one of the following three preferred formats:

- **TIFF:** This is an image made up of pixels and is the most universal and most widely supported format across Windows and Mac platforms. Most graphics packages can save a file as a TIFF. The higher the resolution (i.e. the number of pixels) the sharper the final image.
  - Colour or greyscale photographic images: 300dpi
  - Line art or combination images: 600/900dpi
  - We would recommend using this format for photographic images.
- **EPS**: An EPS is essentially an envelope for holding text and images. Line art can be produced as an EPS (in Illustrator, for example). There are virtually no limits to scaling line art saved as an EPS. It can also contain TIFF images. However, please ensure that all fonts are embedded (that is, saved as outlines) and that line weights are not defined as hairline.
- **PDF**: This format is, again, like an EPS in that it is an envelope for holding different kinds of images and line art. Great care should be taken to ensure that fonts are embedded and that original images are at the correct size and resolution before being saved as a PDF. It is possible to save or export as TIFF or EPS from most graphics applications, just as it is possible to save direct to a PDF from most graphics packages by using a postscript printer driver. PDF creation packages (e.g. Acrobat Distiller) are also now widely available.

Other file formats

- **JPEG**: A JPEG compressed TIFF is acceptable as long as the degree of compression is moderate. It is better to use a JPEG for online images as a good quality image is achievable even with a high degree of compression.
- **GIF**: A format suitable for images that contain few colours. Again, this should only be used for images intended for the web.
- We cannot guarantee the quality of images supplied in other formats.

Colour:

- *Greyscale, CMYK, RGB.*
- **Greyscale** art should be saved in greyscale mode.
- **C**yan**M**agenta**Y**ellow**B**lack are the base colours used during the printing process.
- Any colour that is to appear in print must be in CMYK mode.
- **R**ed**G**reen**B**lue are the colours used by monitors and default scanner settings. Any colour that is to appear online must be in RGB mode.

Guidelines for Math

- Set display equations in MathType. Each display equation should be in its own MathType object. Each MathType object should contain the entire equation, including final punctuation. The equation number should be set as Microsoft Word regular text, outside the MathType object, separated by either a tab or a space.
- Set in-text (inline) math in Microsoft Word regular text. Exception: If in-text (inline) math has elements that should be stacked or have rules, circumflexes, arrows, or other accents spanning over more than one character, set in MathType as ‘Inline Equation.’
- If any characters cannot be found in Word’s Symbol palette (‘(normal text),’ ‘Times New Roman,’ or ‘Symbol’), please set in MathType.
- No display equations are allowed in figure captions, table titles, or table footnotes. If a display equation occurs in a text footnote, it is best to recast it as inline math. There are a few journals with lengthy footnotes with style exceptions to this rule.

- No numbered equations are allowed in table footnotes.
- Display and/or numbered equations ARE allowed in table body, but must be ‘inline’ when converted to MathML equations.

Structure and style of your original research article

The page provides an overview of the structure and style of your original research article to be submitted to the African Vision and Eye Health. An original article provides an overview of innovative research in a particular field within or related to the focus and scope of the journal, presented according to a clear and well-structured format (between 3500 and 7000 words with a maximum of 60 references).

When presenting your article in English. Please use British English, that is, according to the Oxford English Dictionary. Avoid Americanisms (e.g. use ‘s’ and not ‘z’ spellings). Consult the Oxford English Dictionary when in doubt and remember to set your version of Microsoft Word to UK English.

Language: Manuscripts must be written in British English or French.

Line numbers: Insert continuous line numbers.

Font type: Palatino

Symbols font type: Times New Roman

General font size: 12pt

Line spacing: 1.5

Headings: Ensure that formatting for headings is consistent in the manuscript.

First headings: normal case, bold and 14pt

Second headings: normal case, underlined and 14pt

Third headings: normal case, bold and 12pt

Fourth headings: normal case, bold, running-in text and separated by a colon.

Our publication system supports a limited range of formats for text and graphics. Text files can be submitted in the following formats only:

Microsoft Word (.doc): We cannot accept Word 2007 DOCX files. If you have created your manuscript using Word 2007, you must save the document as a Word 2003 file before submission.

Rich Text Format (RTF) documents uploaded during Step 2 of the submission process. Users of other word processing packages should save or convert their files to RTF before uploading. Many free tools are available that will make this process easier.

For full details on how to ensure your manuscript adheres to the house style, click here.

The structure and style of your original article

Page 1

The format of the compulsory cover letter forms part of your submission, is on the first page of your manuscript and should always be presented in English. You should provide all of the following elements:

Full author details: Provide title(s), full name(s), position(s), affiliation(s) and contact details (postal address, email, telephone and cellular number) of each author.

Corresponding author: Identify to whom all correspondence should be addressed.

Summary: Lastly, a list containing the number of words, pages, tables, figures and/or other supplementary material should accompany the submission.

Page 2 and onwards

Title: The article's full title should contain a maximum of 95 characters (including spaces).

Abstract: The abstract, written in English, should be no longer than 250 words and must be written in the past tense. The abstract should give a succinct account of the objectives, methods, results and significance of the matter. The structured abstract for an Original Research article should consist of six paragraphs labelled Background, Aim, Setting, Methods, Results and Conclusion. The journal can translate into French if this is difficult for you.

Background: Summarise the social value (importance, relevance) and scientific value (knowledge gap) that your study addresses.

Aim: State the overall aim of the study.

Setting: State the setting for the study.

Methods: Clearly express the basic design of the study, and name or briefly describe the methods used without going into excessive detail.

Results: State the main findings.

Conclusion: State your conclusion and any key implications or recommendations.

Do not cite references and do not use abbreviations excessively in the abstract.

The following headings serve as a guide for presenting your research in a well-structured original article. As an author you should include all first-level headings, but subsequent headings (second- and third-level headings) can be changed.

Introduction (first-level heading)

The introduction must contain your argument for the social and scientific value of the study, as well as the aim and objectives:

Social value: The first part of the introduction should make a clear and logical argument for the importance or relevance of the study. Your argument should be supported by use of evidence from the literature.

Scientific value: The second part of the introduction should make a clear and logical argument for the originality of the study. This should include a summary of what is already known about the research question or specific topic, and should clarify the knowledge gap that this study will address. Your argument should be supported by use of evidence from the literature.

Conceptual framework: In some research articles it will also be important to describe the underlying theoretical basis for the research and how these theories are linked together in a conceptual framework. The theoretical evidence used to construct the conceptual framework should be referenced from the literature.

Aim and objectives: The introduction should conclude with a clear summary of the aim and objectives of this study.

Research methods and design (first-level heading)

The methods should include:

Study design (second-level heading): An outline of the type of study design.

Setting (second-level heading): A description of the setting for the study; for example, the type of community from which the participants came or the nature of the health system and services in which the study is conducted.

Study population and sampling strategy (second-level heading): Describe the study population and any inclusion or exclusion criteria. Describe the intended sample size and your sample size calculation or justification. Describe the sampling strategy used. Describe in practical terms how this was implemented.

Intervention (if appropriate) (second-level heading): If there were intervention and comparison groups, describe the intervention in detail and what happened to the comparison groups.

Data collection (second-level heading): Define the data collection tools that were used and their validity. Describe in practical terms how data were collected and any key issues involved, e.g. language barriers.

Data analysis (second-level heading): Describe how data were captured, checked and cleaned. Describe the analysis process, for example, the statistical tests used orsteps followed in qualitative data analysis.

Ethical considerations (second-level heading): Approval must have been obtained for all studies from the author's institution or other relevant ethics committee and the institution’s name and permit numbers should be stated here.

Results (first-level heading)

Present the results of your study in a logical sequence that addresses the aim and objectives of your study. Use tables and figures as required to present your findings. Use quotations as required to establish your interpretation of qualitative data.

All units should conform to the SI convention and be abbreviated accordingly. Metric units and their international symbols are used throughout, as is the decimal point (not the decimal comma).

Discussion (first-level heading)

The discussion section should address the following four elements:

Key findings: Summarise the key findings without reiterating details of the results.

Discussion of key findings: Explain how the key findings relate to previous research or to existing knowledge, practice or policy.

Strengths and limitations: Describe the strengths and limitations of your methods and what the reader should take into account when interpreting your results.

Implications or recommendations: State the implications of your study or recommendations for future research (questions that remain unanswered), policy or practice. Make sure that the recommendations flow directly from your findings.

Conclusion (first-level heading)

Provide a brief conclusion that summarises the results and their meaning or significance in relation to each objective of the study.

Acknowledgements (first-level heading)

If, through your study, you received any significant help in conceiving, designing or carrying out the work, or received materials from someone who did you a favour by supplying them, you must acknowledge their assistance and the service or material provided. Authors should

always acknowledge outside reviewers of their drafts and any sources of funding that supported the research.

Competing interests (second-level heading): A competing interest exists when your interpretation of data or presentation of information may be influenced by your personal or financial relationship with other people or organisations that can potentially prevent you from executing and publishing unbiased research. Authors should disclose any financial competing interests but also any non-financial competing interests that may cause them embarrassment were they to become public after the publication of the manuscript. Where an author has no such competing interests, the listing will read as follows: ‘The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.’

Authors' contributions (second-level heading): This section is necessary to give appropriate credit to each author, and to the authors' applicable institution. The individual contributions of authors should be specified with their affiliation at the time of the study and completion of the work. An ‘author’ is generally considered to be someone who has made substantive intellectual contributions to a published study. Contributions made by each of the authors listed can follow the example below (please note the use of authors’ initials):

J.K. (University of Pretoria) was the project leader, L.M.N. (University of KwaZulu-Natal) and A.B. (Stellenbosch University) were responsible for experimental and project design. L.M.N. performed most of the experiments. P.R. (Cape Peninsula University of Technology) made conceptual contributions and S.T. (University of Cape Town), U.V. (University of Cape Town) and C.D. (University of Cape Town) performed some of the experiments. S.M. (Cape Peninsula University of Technology) and V.C. (Cape Peninsula University of Technology) prepared the samples and calculations were performed by C.S. (Cape Peninsula University of Technology).

References (first-level heading)

Begin the reference list on a separate page, and give no more than 60 references in all. The African Vision and Eye Health uses the Vancouver referencing style, details of which can be downloaded from the journal website. Note: No other style will be permitted.

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

16 October 2015

Dr Etienne Phillip LE Roux
405 Umhlanga Rocks
Durban
4320
epleroux@yahoo.com

PROTOCOL: Bevacizumabin a treat-and-extend regimen for neovascular age related macular degeneration in a resource limited. School of Clinical Medicine: MMed 213574210.
BREC REF: BE017/15

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 13 January 2015.

The study was provisionally approved pending appropriate responses to queries raised. Your responses received on 08 October 2015 to queries raised on 06 October 2015 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have been met and the study is given full ethics approval.

This approval is valid for one year from **16 October 2015.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its meeting taking place on **10 November 2015.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor J Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

cc: ruraleye@gmail.com

health

Department:
Health
PROVINCE OF KWAZULU-NATAL

Inkosi Albert Luthuli Central Hospital
Ethekwini Health District
Office of the Medical Manager
Private Bag X 03, Mayville, 4058
800 Bellair Road, Mayville, 4058
Tel.: 031 240 1059,
Fax.: 031 240 1050
Email.:ursulanun@ialch.co.za
www.kznhealth.gov.za

1 July 2015

Dr E P Le Roux
Department of Opthalmology
IALCH

Dear Dr Roux

Re: Approved Research: Ref No: BE017/15: Bevacizumabin a treat-and extend regimen for neovascular age related macular degeneration in a resource limited.

As per the policy of the Provincial Health Research Committee (PHRC), you are hereby granted permission to conduct the above mentioned research once all relevant documentation has been submitted to PHRC inclusive of Full Ethical Approval.

Kindly note the following.

1. The research should adhere to all policies, procedures, protocols and guidelines of the KwaZulu-Natal Department of Health.
2. Research will only commence once the PHRC has granted approval to the researcher.
3. The researcher must ensure that the Medical Manager is informed before the commencement of the research by means of the approval letter by the chairperson of the PHRC.
4. The Medical Manager expects to be provided feedback on the findings of the research.
5. Kindly submit your research to:

   The Secretariat
   Health Research & Knowledge Management
   330 Langaliballe Street, Pietermaritzburg, 3200
   Private Bag X9501, Pietermaritzburg, 3201
   Tel: 033395-3123, Fax 033394-3782
   Email: hrkm@kznhealth.gov.za

Yours faithfully

.....

Dr M Letebele
Medical Manager

health

Department:
Health
PROVINCE OF KWAZULU-NATAL

Inkosi Albert Luthuli Central Hospital
Ethekwini Health District
Office of the Medical Manager
Private Bag X 03, Mayville, 4058
800 Bellair Road, Mayville, 4058
Tel.: 031 240 1059,
Fax.: 031 240 1050
Email.:ursulanun@ialch.co.za
www.kznhealth.gov.za

Reference: BE017/15
Enquiries: Medical Management

2 July 2015

Dr E P Le Roux
Department of Ophthalmology
IALCH

Dear Dr Le Roux

RE: PERMISSION TO CONDUCT RESEARCH AT IALCH

I have pleasure in informing you that permission has been granted to you by the Medical Manager to conduct research on: **Bevacizumabin a treat-and extend regimen for neovascular age related macular degeneration in a resource limited.**

Kindly take note of the following information before you continue:

1. Please ensure that you adhere to all the policies, procedures, protocols and guidelines of the Department of Health with regards to this research.
2. This research will only commence once this office has received confirmation from the Provincial Health Research Committee in the KZN Department of Health.
3. Kindly ensure that this office is informed before you commence your research.
4. The hospital will not provide any resources for this research.
5. You will be expected to provide feedback once your research is complete to the Medical Manager.

Yours faithfully

.....
Dr M Letebele
Medical Manager

PERMISSION TO CONDUCT A RESEARCH STUDY/TRIAL

This must be completed and submitted to the Medical Superintendent/s / Hospital Manager/s for signature.

For **King Edward VIII Hospital (KEH) and Inkosi Albert Luthuli Central Hospital (IALCH)** studies please submit the document together with the following:

1. Research proposal and protocol.
2. Letter giving provisional ethical approval.
3. Details of other research presently being performed by yourself if in the employ of KEH, (individually or as a collaborator).
4. Details of any financial or human resource implications to KEH, including all laboratory tests, EEGs, X-rays, use of nurses, etc. (See Addendum 1)
5. Declaration of all funding applications / grants, please supply substantiating documentation.
6. Complete the attached KEH Form - *"Research Details"*

Once the document has been signed it should be returned to **Mrs Patricia Ngwenya:** at the Biomedical Research Ethics Administration, Room N40, Govan Mbeki Building, Westville Campus, University of KwaZulu-Natal.

To: Chief Medical Superintendent / Hospital Manager

Permission is requested to conduct the above research study at the hospital/s indicated below:

Site 1 address:

IALCH

Investigator/s:

Principal: DR E. P. LERCUX

Co-investigator: /

Co-Investigator: /

Signature of Chief Medical Superintendent/Hospital Manager:

Date: 15/07/2014

Site 2 address:

Investigator/s

Principal: _____

Co-investigator: _____

Co-Investigator: _____

Signature of Chief Medical Superintendent / Hospital Manager:

Date: _____

NB: Medical Superintendent/s / Hospital Manager/s to send a copy of this document to Natalia

```
GET
  FILE='E:\2016_MFK\Konsultasie\Ander\EttienneLeRoux20161121.sav'.

Warning # 5281.  Command name: GET FILE
SPSS Statistics is running in Unicode encoding mode.  This file is encoded in
a locale-specific (code page) encoding.  The defined width of any string
variables are automatically tripled in order to avoid possible data loss.  You
can use ALTER TYPE to set the width of string variables to the width of the
longest observed value for each string variable.
ALTER TYPE ALL(A=AMIN).
```

Alter Type

Notes

Output Created		27-NOV-2016 18:22:33
Comments		
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Syntax		ALTER TYPE ALL(A=AMIN).
Resources	Processor Time	00:00:00.00
	Elapsed Time	00:00:00.66

E:\2016_MFK\Konsultasie\Ander\EttienneLeRoux20161121.sav

Altered Types

PatientNo	A6	AMIN
SEX	A3	AMIN
RACE	A3	AMIN
OD_OS	A6	AMIN
Year1_1	A3	AMIN
Year1_5	A6	AMIN

```
DATASET NAME DataSet1 WINDOW=FRONT.
FREQUENCIES VARIABLES=FollowUp
  /ORDER=ANALYSIS.
```

Frequencies

```
AGE CODE 1=Younger than 70
2=Between 70 and 74
3=Between 75 and 79
4=Between 80 and 84
5=85 and older
```

Notes

Output Created		27-NOV-2016 18:25:56
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Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data.
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	Elapsed Time	00:00:00.03

[DataSet1] E:\2016_MFK\Konsultasie\Ander\EttienneLeRoux20161121.sav

Statistics

FollowUp

N	Valid	53

	Missing	0

FollowUp

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1.0	29	54.7	54.7	54.7
	2.0	24	45.3	45.3	100.0
	Total	53	100.0	100.0	

```
FREQUENCIES VARIABLES=AGE Age_code SEX gender_Code RACE Race_code OD_OS OD_OS_Code
  /ORDER=ANALYSIS.
```

Frequencies

Notes

Output Created		27-NOV-2016 18:27:21
Comments		
Input	Data	E:\2016_MFK\Konsultasie\Ander\EttienneLeRoux20161121.sav
	Active Dataset	DataSet1
	Filter	<none>

	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	53
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data.
Syntax		FREQUENCIES VARIABLES=AGE Age_code SEX gender_Code RACE Race_code OD_OS OD_OS_Code /ORDER=ANALYSIS.
Resources	Processor Time	00:00:00.00
	Elapsed Time	00:00:00.00

Statistics

		AGE	Age_code	SEX	gender_Code	RACE	Race_code		
N	Valid	53	53	53	53	53	53		
	Missing	0	0	0	0	0	0		

Statistics

		OD_OS	OD_OS_Code
N	Valid	53	53
	Missing	0	0

Frequency Table

AGE

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	61	1	1.9	1.9	1.9
	62	1	1.9	1.9	3.8
	68	2	3.8	3.8	7.5
	71	2	3.8	3.8	11.3
	72	3	5.7	5.7	17.0
	73	3	5.7	5.7	22.6
	74	1	1.9	1.9	24.5
	75	5	9.4	9.4	34.0
	76	3	5.7	5.7	39.6
	77	2	3.8	3.8	43.4
	79	3	5.7	5.7	49.1
	80	3	5.7	5.7	54.7
	81	2	3.8	3.8	58.5
	82	2	3.8	3.8	62.3
	83	5	9.4	9.4	71.7
	84	4	7.5	7.5	79.2

	86	7	13.2	13.2	92.5
	89	1	1.9	1.9	94.3
	90	1	1.9	1.9	96.2
	93	2	3.8	3.8	100.0
	Total	53	100.0	100.0	

Age_code

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1.0	4	7.5	7.5	7.5
	2.0	9	17.0	17.0	24.5
	3.0	13	24.5	24.5	49.1
	4.0	16	30.2	30.2	79.2
	5.0	11	20.8	20.8	100.0
	Total	53	100.0	100.0	

SEX

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	F	24	45.3	45.3	45.3
	M	29	54.7	54.7	100.0
	Total	53	100.0	100.0	

Gender Code :
1 = Female
2 = Male

gender_Code

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1.0	24	45.3	45.3	45.3
	2.0	29	54.7	54.7	100.0
	Total	53	100.0	100.0	

RACE

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	B	3	5.7	5.7	5.7
	I	8	15.1	15.1	20.8
	W	42	79.2	79.2	100.0
	Total	53	100.0	100.0	

Race Code:
1 = Black
2 = Indian
3 = White

Race_code

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	3	5.7	5.7	5.7
	2	8	15.1	15.1	20.8
	3	42	79.2	79.2	100.0
	Total	53	100.0	100.0	

OD_OS

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	OD	31	58.5	58.5	58.5
	OS	22	41.5	41.5	100.0
	Total	53	100.0	100.0	

OD-OS_Code
1= OD
2 = OS

OD_OS_Code

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1.0	31	58.5	58.5	58.5
	2.0	22	41.5	41.5	100.0
	Total	53	100.0	100.0	

```
DESCRIPTIVES VARIABLES=VA_Start VA_1year VA_2year
  /STATISTICS=MEAN STDDEV MIN MAX KURTOSIS SKEWNESS.
```

Descriptives

Notes

Output Created		27-NOV-2016 18:30:37
Comments		
Input	Data	E:\2016_MFK\Konsultasie\Ander\EttienneLeRoux20161121.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	53
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	All non-missing data are used.
Syntax		DESCRIPTIVES VARIABLES=VA_Start VA_1year VA_2year /STATISTICS=MEAN STDDEV MIN MAX KURTOSIS SKEWNESS.
Resources	Processor Time	00:00:00.00
	Elapsed Time	00:00:00.00

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Skewness			
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error		
VA_Start	53	.0	.8	.213	.1743	1.637	.327		
VA_1year	53	.1	1.0	.383	.2673	.846	.327		
VA_2year	24	.10	1.00	.4479	.26600	.519	.472		
Valid N (listwise)	24								

Descriptive Statistics

	Kurtosis	
	Statistic	Std. Error
VA_Start	3.229	.644
VA_1year	-.083	.644
VA_2year	-1.011	.918
Valid N (listwise)		

```
DESCRIPTIVES VARIABLES=CMT_Start CMT_1year CMT_2year
  /STATISTICS=MEAN STDDEV MIN MAX KURTOSIS SKEWNESS.
```

Descriptives

Notes

Output Created		27-NOV-2016 18:31:01
Comments		
Input	Data	E:\2016_MFK\Konsultasie\Ander\EttienneLeRoux20161121.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	53
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	All non-missing data are used.
Syntax		DESCRIPTIVES VARIABLES=CMT_Start CMT_1year CMT_2year /STATISTICS=MEAN STDDEV MIN MAX KURTOSIS SKEWNESS.
Resources	Processor Time	00:00:00.02
	Elapsed Time	00:00:00.03

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Skewness			
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error		
CMT_Start	48	215.0	611.0	333.458	85.4430	1.160	.343		
CMT_1year	48	134.0	774.0	265.354	93.5554	3.528	.343		
CMT_2year	24	172.0	349.0	245.792	49.7734	.494	.472		
Valid N (listwise)	24								

Descriptive Statistics

	Kurtosis	
	Statistic	Std. Error
CMT_Start	1.470	.674
CMT_1year	18.316	.674
CMT_2year	-.358	.918
Valid N (listwise)		

Average time between injections during Year 1

```
DESCRIPTIVES VARIABLES=BetweenTime_1
  /STATISTICS=MEAN STDDEV MIN MAX KURTOSIS SKEWNESS.
```

Descriptives

Notes

Output Created		27-NOV-2016 18:33:19
Comments		
Input	Data	E:\2016_MFK\Konsultasie\Ander\EttienneLeRoux20161121.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	53
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	All non-missing data are used.
Syntax		DESCRIPTIVES VARIABLES=BetweenTime_1 /STATISTICS=MEAN STDDEV MIN MAX KURTOSIS SKEWNESS.
Resources	Processor Time	00:00:00.00
	Elapsed Time	00:00:00.00

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Statistic
BetweenTime_1	53	3.90909090909090	9.25000000000000	5.591832747964824	1.18094289084597
Valid N (listwise)	53				

Descriptive Statistics

	Skewness		Kurtosis	
	Statistic	Std. Error	Statistic	Std. Error
BetweenTime_1	1.202	.327	1.766	.644
Valid N (listwise)				

Average time between injections in Year 2

```
DESCRIPTIVES VARIABLES=BetweenTime_2
  /STATISTICS=MEAN STDDEV MIN MAX KURTOSIS SKEWNESS.
```

Descriptives

Notes

Output Created		27-NOV-2016 18:34:06
Comments		
Input	Data	E:\2016_MFK\Konsultasie\Ander\EttienneLeRoux20161121.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	53
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	All non-missing data are used.
Syntax		DESCRIPTIVES VARIABLES=BetweenTime_2 /STATISTICS=MEAN STDDEV MIN MAX KURTOSIS SKEWNESS.
Resources	Processor Time	00:00:00.00
	Elapsed Time	00:00:00.03

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Statistic
BetweenTime_2	25	4.000000000000000	14.500000000000000	8.582634920634922	3.129739662351875
Valid N (listwise)	25				

Descriptive Statistics

	Skewness		Kurtosis	
	Statistic	Std. Error	Statistic	Std. Error
BetweenTime_2	.312	.464	-1.166	.902
Valid N (listwise)				

Average time between injections

```
COMPUTE TimeBetweenInj=Mean(Year1_1,Year1_2,Year1_3,Year1_4,Year1_5,Year1_6,Year1_7,Year1_8,Year1_9,
    Year1_10,Year1_11,Year1_12,Year2_1,Year2_2,Year2_3,Year2_4,Year2_5,Year2_6,Year2_7,Year2_8,Year2_9,
    Year2_10).
EXECUTE.
DESCRIPTIVES VARIABLES=TimeBetweenInj
  /STATISTICS=MEAN STDDEV MIN MAX KURTOSIS SKEWNESS.
```

Descriptives

Notes

Output Created		27-NOV-2016 18:37:24
Comments		
Input	Data	E:\2016_MFK\Konsultasie\Ander\EttienneLeRoux20161121.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	53
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	All non-missing data are used.
Syntax		DESCRIPTIVES VARIABLES=TimeBetweenInj /STATISTICS=MEAN STDDEV MIN MAX KURTOSIS SKEWNESS.
Resources	Processor Time	00:00:00.02
	Elapsed Time	00:00:00.02

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Skewness			
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error		
TimeBetweenInj	53	3.92	10.20	6.0373	1.43582	.956	.327		
Valid N (listwise)	53								

Descriptive Statistics

	Kurtosis	
	Statistic	Std. Error
TimeBetweenInj	.409	.644
Valid N (listwise)		

```
FREQUENCIES VARIABLES=Year1_1 Year1_2 Year1_3 Year1_4 Year1_5 Year1_6 Year1_7 Year1_8 Year1_9
    Year1_10 Year1_11 Year1_12 Year2_1 Year2_2 Year2_3 Year2_4 Year2_5 Year2_6 Year2_7 Year2_8 Year2_9
    Year2_10
  /STATISTICS=STDDEV MINIMUM MAXIMUM MEAN MEDIAN SKEWNESS SESKEW KURTOSIS SEKURT
  /ORDER=ANALYSIS.
```

Frequencies

Notes

Output Created		27-NOV-2016 18:38:59
Comments		
Input	Data	E:\2016_MFK\Konsultasie\Ander\EttienneLeRoux20161121.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	53
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data.

Syntax		FREQUENCIES VARIABLES=Year1_1 Year1_2 Year1_3 Year1_4 Year1_5 Year1_6 Year1_7 Year1_8 Year1_9 Year1_10 Year1_11 Year1_12 Year2_1 Year2_2 Year2_3 Year2_4 Year2_5 Year2_6 Year2_7 Year2_8 Year2_9 Year2_10 /STATISTICS=STDDEV MINIMUM MAXIMUM MEAN MEDIAN SKEWNESS SESKEW KURTOSIS SEKURT /ORDER=ANALYSIS.
Resources	Processor Time	00:00:00.00
	Elapsed Time	00:00:00.03

		Year1_1	Year1_2	Year1_3	Year1_4	Year1_5	Year1_6	Year1_7
N	Valid	53	53	53	52	51	49	45
	Missing	0	0	0	1	2	4	8
Mean		4.302	4.113	4.962	5.885	5.863	6.490	6.289
Median		4.000	4.000	4.000	4.000	6.000	6.000	6.000
Std. Deviation		1.0112	.5771	2.1390	4.1000	2.1356	2.3728	2.3122
Skewness		4.105	3.123	3.322	3.764	1.250	.857	.531
Std. Error of Skewness		.327	.327	.327	.330	.333	.340	.354
Kurtosis		17.133	13.754	13.683	15.233	1.114	.358	-.881
Std. Error of Kurtosis		.644	.644	.644	.650	.656	.668	.695
Minimum		4.0	3.0	3.0	4.0	4.0	4.0	4.0
Maximum		9.0	7.0	16.0	26.0	12.0	13.0	12.0

		Year1_8	Year1_9	Year1_10	Year1_11	Year1_12	Year2_1
N	Valid	39	28	19	12	4	24
	Missing	14	25	34	41	49	29
Mean		6.000	4.929	5.526	4.667	4.250	8.542
Median		5.000	4.000	4.000	4.000	4.000	8.000
Std. Deviation		2.4170	1.1841	2.0915	.9847	.5000	2.7184
Skewness		1.155	1.012	1.282	.812	2.000	.064
Std. Error of Skewness		.378	.441	.524	.637	1.014	.472
Kurtosis		.307	.045	.896	-1.650	4.000	-.609
Std. Error of Kurtosis		.741	.858	1.014	1.232	2.619	.918

Minimum	4.0	4.0	4.0	4.0	4.0	4.0
Maximum	12.0	8.0	11.0	6.0	5.0	14.0

		Year2_2	Year2_3	Year2_4	Year2_5	Year2_6	Year2_7	Year2_8
N	Valid	23	22	19	16	11	11	9
	Missing	30	31	34	37	42	42	44
Mean		7.826	9.182	7.895	8.250	6.182	5.909	4.778
Median		7.000	9.500	7.000	8.000	6.000	5.000	4.000
Std. Deviation		3.4726	3.5675	3.9426	3.3367	1.8878	2.1659	1.2019
Skewness		.374	.092	.677	.083	.663	.503	1.093
Std. Error of Skewness		.481	.491	.524	.564	.661	.661	.717
Kurtosis		-1.217	-1.076	-.836	-1.679	.199	-1.768	-.586
Std. Error of Kurtosis		.935	.953	1.014	1.091	1.279	1.279	1.400
Minimum		4.0	4.0	4.0	4.0	4.0	4.0	4.0
Maximum		14.0	16.0	16.0	13.0	10.0	9.0	7.0

Statistics

		Year2_9	Year2_10
N	Valid	7	3
	Missing	46	50
Mean		5.571	4.667
Median		6.000	4.000
Std. Deviation		1.6183	1.1547
Skewness		.317	1.732
Std. Error of Skewness		.794	1.225
Kurtosis		-1.501	

Std. Error of Kurtosis	1.587	
Minimum	4.0	4.0
Maximum	8.0	6.0

Frequency Table

Year1_1

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	46	86.8	86.8	86.8
	5.0	4	7.5	7.5	94.3
	6.0	1	1.9	1.9	96.2
	9.0	2	3.8	3.8	100.0
	Total	53	100.0	100.0	

Year1_2

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	3.0	2	3.8	3.8	3.8
	4.0	46	86.8	86.8	90.6
	5.0	3	5.7	5.7	96.2
	6.0	1	1.9	1.9	98.1
	7.0	1	1.9	1.9	100.0
	Total	53	100.0	100.0	

Year1_3

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	3.0	1	1.9	1.9	1.9
	4.0	36	67.9	67.9	69.8
	5.0	2	3.8	3.8	73.6
	6.0	9	17.0	17.0	90.6
	8.0	2	3.8	3.8	94.3
	10.0	2	3.8	3.8	98.1
	16.0	1	1.9	1.9	100.0
	Total	53	100.0	100.0	

Year1_4

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	27	50.9	51.9	51.9
	5.0	8	15.1	15.4	67.3
	6.0	7	13.2	13.5	80.8
	7.0	2	3.8	3.8	84.6
	8.0	5	9.4	9.6	94.2
	14.0	1	1.9	1.9	96.2
	22.0	1	1.9	1.9	98.1
	26.0	1	1.9	1.9	100.0
	Total	52	98.1	100.0	
Missing	System	1	1.9		
Total		53	100.0		

Year1_5

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	20	37.7	39.2	39.2
	5.0	5	9.4	9.8	49.0
	6.0	13	24.5	25.5	74.5
	7.0	2	3.8	3.9	78.4
	8.0	5	9.4	9.8	88.2
	9.0	2	3.8	3.9	92.2
	10.0	2	3.8	3.9	96.1
	12.0	2	3.8	3.9	100.0
	Total	51	96.2	100.0	
Missing	System	2	3.8		
Total		53	100.0		

Year1_6

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	16	30.2	32.7	32.7
	5.0	1	1.9	2.0	34.7
	6.0	11	20.8	22.4	57.1
	7.0	4	7.5	8.2	65.3
	8.0	11	20.8	22.4	87.8
	10.0	3	5.7	6.1	93.9
	12.0	2	3.8	4.1	98.0
	13.0	1	1.9	2.0	100.0

	Total	49	92.5	100.0	
Missing	System	4	7.5		
Total		53	100.0		

Year1_7

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	18	34.0	40.0	40.0
	5.0	2	3.8	4.4	44.4
	6.0	6	11.3	13.3	57.8
	7.0	2	3.8	4.4	62.2
	8.0	9	17.0	20.0	82.2
	9.0	3	5.7	6.7	88.9
	10.0	4	7.5	8.9	97.8
	12.0	1	1.9	2.2	100.0
	Total	45	84.9	100.0	
Missing	System	8	15.1		
Total		53	100.0		

Year1_8

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	16	30.2	41.0	41.0
	5.0	5	9.4	12.8	53.8
	6.0	7	13.2	17.9	71.8
	7.0	1	1.9	2.6	74.4
	8.0	4	7.5	10.3	84.6
	10.0	4	7.5	10.3	94.9
	12.0	2	3.8	5.1	100.0
	Total	39	73.6	100.0	
Missing	System	14	26.4		
Total		53	100.0		

Year1_9

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	15	28.3	53.6	53.6
	5.0	4	7.5	14.3	67.9
	6.0	6	11.3	21.4	89.3
	7.0	2	3.8	7.1	96.4
	8.0	1	1.9	3.6	100.0
	Total	28	52.8	100.0	
Missing	System	25	47.2		
Total		53	100.0		

Year1_10

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	10	18.9	52.6	52.6
	5.0	2	3.8	10.5	63.2
	6.0	2	3.8	10.5	73.7
	8.0	4	7.5	21.1	94.7
	11.0	1	1.9	5.3	100.0
	Total	19	35.8	100.0	
Missing	System	34	64.2		
Total		53	100.0		

Year1_11

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	8	15.1	66.7	66.7
	6.0	4	7.5	33.3	100.0
	Total	12	22.6	100.0	
Missing	System	41	77.4		
Total		53	100.0		

Year1_12

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	3	5.7	75.0	75.0
	5.0	1	1.9	25.0	100.0
	Total	4	7.5	100.0	
Missing	System	49	92.5		
Total		53	100.0		

Year2_1

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	2	3.8	8.3	8.3
	5.0	2	3.8	8.3	16.7
	6.0	2	3.8	8.3	25.0
	7.0	1	1.9	4.2	29.2
	8.0	6	11.3	25.0	54.2
	9.0	2	3.8	8.3	62.5
	10.0	4	7.5	16.7	79.2
	12.0	4	7.5	16.7	95.8
	14.0	1	1.9	4.2	100.0
	Total	24	45.3	100.0	
Missing	System	29	54.7		
Total		53	100.0		

Year2_2

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	7	13.2	30.4	30.4
	6.0	4	7.5	17.4	47.8
	7.0	1	1.9	4.3	52.2
	8.0	1	1.9	4.3	56.5
	9.0	1	1.9	4.3	60.9
	10.0	4	7.5	17.4	78.3
	12.0	3	5.7	13.0	91.3
	14.0	2	3.8	8.7	100.0
	Total	23	43.4	100.0	
Missing	System	30	56.6		
Total		53	100.0		

Year2_3

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	3	5.7	13.6	13.6
	6.0	4	7.5	18.2	31.8
	7.0	2	3.8	9.1	40.9
	8.0	1	1.9	4.5	45.5
	9.0	1	1.9	4.5	50.0
	10.0	1	1.9	4.5	54.5
	11.0	3	5.7	13.6	68.2
	12.0	4	7.5	18.2	86.4
	14.0	2	3.8	9.1	95.5

	16.0	1	1.9	4.5	100.0
	Total	22	41.5	100.0	
Missing	System	31	58.5		
Total		53	100.0		

Year2_4

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	6	11.3	31.6	31.6
	5.0	1	1.9	5.3	36.8
	6.0	2	3.8	10.5	47.4
	7.0	1	1.9	5.3	52.6
	8.0	3	5.7	15.8	68.4
	11.0	1	1.9	5.3	73.7
	12.0	2	3.8	10.5	84.2
	13.0	1	1.9	5.3	89.5
	14.0	1	1.9	5.3	94.7
	16.0	1	1.9	5.3	100.0
	Total	19	35.8	100.0	
Missing	System	34	64.2		
Total		53	100.0		

Year2_5

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	3	5.7	18.8	18.8
	5.0	1	1.9	6.3	25.0
	6.0	3	5.7	18.8	43.8
	8.0	2	3.8	12.5	56.3
	9.0	1	1.9	6.3	62.5
	11.0	1	1.9	6.3	68.8
	12.0	4	7.5	25.0	93.8
	13.0	1	1.9	6.3	100.0
	Total	16	30.2	100.0	
Missing	System	37	69.8		
Total		53	100.0		

Year2_6

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	3	5.7	27.3	27.3
	6.0	5	9.4	45.5	72.7
	8.0	2	3.8	18.2	90.9
	10.0	1	1.9	9.1	100.0
	Total	11	20.8	100.0	
Missing	System	42	79.2		
Total		53	100.0		

Year2_7

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	5	9.4	45.5	45.5
	5.0	1	1.9	9.1	54.5
	6.0	1	1.9	9.1	63.6
	8.0	2	3.8	18.2	81.8
	9.0	2	3.8	18.2	100.0
	Total	11	20.8	100.0	
Missing	System	42	79.2		
Total		53	100.0		

Year2_8

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	6	11.3	66.7	66.7
	6.0	2	3.8	22.2	88.9
	7.0	1	1.9	11.1	100.0
	Total	9	17.0	100.0	
Missing	System	44	83.0		
Total		53	100.0		

Year2_9

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	3	5.7	42.9	42.9
	6.0	2	3.8	28.6	71.4
	7.0	1	1.9	14.3	85.7
	8.0	1	1.9	14.3	100.0
	Total	7	13.2	100.0	
Missing	System	46	86.8		
Total		53	100.0		

Year2_10

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	4.0	2	3.8	66.7	66.7
	6.0	1	1.9	33.3	100.0
	Total	3	5.7	100.0	
Missing	System	50	94.3		
Total		53	100.0		

```
T-TEST PAIRS=VA_Start VA_Start VA_1year WITH VA_1year VA_2year VA_2year (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Notes

Output Created		27-NOV-2016 18:39:57
Comments		
Input	Data	E:\2016_MFK\Konsultasie\Ander\EttienneLeRoux20161121.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	53
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.

Syntax		T-TEST PAIRS=VA_Start VA_Start VA_1year WITH VA_1year VA_2year VA_2year (PAIRED) /CRITERIA=CI(.9500) /MISSING=ANALYSIS.
Resources	Processor Time	00:00:00.00
	Elapsed Time	00:00:00.03

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	VA_Start	.213	53	.1743	.0239
	VA_1year	.383	53	.2673	.0367
Pair 2	VA_Start	.269	24	.2100	.0429
	VA_2year	.4479	24	.26600	.05430
Pair 3	VA_1year	.442	24	.2773	.0566
	VA_2year	.4479	24	.26600	.05430

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	VA_Start & VA_1year	53	.310	.024
Pair 2	VA_Start & VA_2year	24	.114	.597
Pair 3	VA_1year & VA_2year	24	.569	.004

		Paired Differences			
					95% Confidence Interval of the Difference
		Mean	Std. Deviation	Std. Error Mean	Lower
Pair 1	VA_Start - VA_1year	-.1704	.2701	.0371	-.2448
Pair 2	VA_Start - VA_2year	-.17917	.31962	.06524	-.31413
Pair 3	VA_1year - VA_2year	-.00625	.25251	.05154	-.11288

Paired Samples Test

		Paired Differences			
		95% Confidence Interval of the Difference			
		Upper	t	df	Sig. (2-tailed)
Pair 1	VA_Start - VA_1year	-.0959	-4.592	52	.000
Pair 2	VA_Start - VA_2year	-.04420	-2.746	23	.012
Pair 3	VA_1year - VA_2year	.10038	-.121	23	.905

```
T-TEST PAIRS=CMT_Start CMT_Start CMT_1year WITH CMT_1year CMT_2year CMT_2year (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Notes

Output Created		27-NOV-2016 18:40:31
Comments		
Input	Data	E:\2016_MFK\Konsultasie\Ander\EttienneLeRoux20161121.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	53
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST PAIRS=CMT_Start CMT_Start CMT_1year WITH CMT_1year CMT_2year CMT_2year (PAIRED) /CRITERIA=CI(.9500) /MISSING=ANALYSIS.
Resources	Processor Time	00:00:00.00

Elapsed Time	00:00:00.01

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	CMT_Start	333.458	48	85.4430	12.3326
	CMT_1year	265.354	48	93.5554	13.5036
Pair 2	CMT_Start	325.458	24	79.0305	16.1320
	CMT_2year	245.792	24	49.7734	10.1599
Pair 3	CMT_1year	251.667	24	51.7961	10.5728
	CMT_2year	245.792	24	49.7734	10.1599

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	CMT_Start & CMT_1year	48	.485	.000
Pair 2	CMT_Start & CMT_2year	24	.521	.009
Pair 3	CMT_1year & CMT_2year	24	.897	.000

		Paired Differences			
					95% Confidence Interval of the Difference
		Mean	Std. Deviation	Std. Error Mean	Lower
Pair 1	CMT_Start - CMT_1year	68.1042	91.1273	13.1531	41.6436
Pair 2	CMT_Start - CMT_2year	79.6667	67.9851	13.8774	50.9591
Pair 3	CMT_1year - CMT_2year	5.8750	23.0977	4.7148	-3.8783

Paired Samples Test

		Paired Differences			
		95% Confidence Interval of the Difference			
		Upper	t	df	Sig. (2-tailed)
Pair 1	CMT_Start - CMT_1year	94.5648	5.178	47	.000
Pair 2	CMT_Start - CMT_2year	108.3742	5.741	23	.000
Pair 3	CMT_1year - CMT_2year	15.6283	1.246	23	.225

```
CORRELATIONS
  /VARIABLES=VA_Start VA_1year VA_2year BetweenTime_1 BetweenTime_2
  /PRINT=TWOTAIL NOSIG
  /MISSING=PAIRWISE.
```

Correlations

Notes

Output Created		27-NOV-2016 18:42:10
Comments		
Input	Data	E:\2016_MFK\Konsultasie\Ander\EttienneLeRoux20161121.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	53
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
Syntax		CORRELATIONS /VARIABLES=VA_Start VA_1year VA_2year BetweenTime_1 BetweenTime_2 /PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.
Resources	Processor Time	00:00:00.00
	Elapsed Time	00:00:00.01

		VA_Start	VA_1year	VA_2year	BetweenTime_1
VA_Start	Pearson Correlation	1	.310*	.114	.451**
	Sig. (2-tailed)		.024	.597	.001
	N	53	53	24	53
VA_1year	Pearson Correlation	.310*	1	.569**	.160
	Sig. (2-tailed)	.024		.004	.251
	N	53	53	24	53
VA_2year	Pearson Correlation	.114	.569**	1	.179
	Sig. (2-tailed)	.597	.004		.402
	N	24	24	24	24
BetweenTime_1	Pearson Correlation	.451**	.160	.179	1
	Sig. (2-tailed)	.001	.251	.402	
	N	53	53	24	53
BetweenTime_2	Pearson Correlation	.392	.230	.168	.660**
	Sig. (2-tailed)	.053	.269	.434	.000
	N	25	25	24	25

Correlations

		BetweenTime_2
VA_Start	Pearson Correlation	.392
	Sig. (2-tailed)	.053
	N	25
VA_1year	Pearson Correlation	.230
	Sig. (2-tailed)	.269

	N	25
VA_2year	Pearson Correlation	.168
	Sig. (2-tailed)	.434
	N	24
BetweenTime_1	Pearson Correlation	.660**
	Sig. (2-tailed)	.000
	N	25
BetweenTime_2	Pearson Correlation	1
	Sig. (2-tailed)	
	N	25

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

```
CORRELATIONS
  /VARIABLES=CMT_Start CMT_1year CMT_2year BetweenTime_1 BetweenTime_2
  /PRINT=TWOTAIL NOSIG
  /MISSING=PAIRWISE.
```

Correlations

Notes

Output Created	27-NOV-2016 18:42:49
Comments	

Input	Data	E:\2016_MFK\Konsultasie\Ander\EttienneLeRoux20161121.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	53
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
Syntax		CORRELATIONS /VARIABLES=CMT_Start CMT_1year CMT_2year BetweenTime_1 BetweenTime_2 /PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.
Resources	Processor Time	00:00:00.02
	Elapsed Time	00:00:00.04

		CMT_Start	CMT_1year	CMT_2year	BetweenTime_1
CMT_Start	Pearson Correlation	1	.485**	.521**	-.261
	Sig. (2-tailed)		.000	.009	.073
	N	48	48	24	48
CMT_1year	Pearson Correlation	.485**	1	.897**	-.284
	Sig. (2-tailed)	.000		.000	.050
	N	48	48	24	48
CMT_2year	Pearson Correlation	.521**	.897**	1	-.236
	Sig. (2-tailed)	.009	.000		.267
	N	24	24	24	24
BetweenTime_1	Pearson Correlation	-.261	-.284	-.236	1
	Sig. (2-tailed)	.073	.050	.267	
	N	48	48	24	53
BetweenTime_2	Pearson Correlation	-.425*	-.431*	-.260	.660**
	Sig. (2-tailed)	.034	.031	.221	.000
	N	25	25	24	25

Correlations

		BetweenTime_2
CMT_Start	Pearson Correlation	-.425*
	Sig. (2-tailed)	.034
	N	25
CMT_1year	Pearson Correlation	-.431*
	Sig. (2-tailed)	.031
	N	25
CMT_2year	Pearson Correlation	-.260

	Sig. (2-tailed)	.221
	N	24
BetweenTime_1	Pearson Correlation	.660**
	Sig. (2-tailed)	.000
	N	25
BetweenTime_2	Pearson Correlation	1
	Sig. (2-tailed)	
	N	25

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

```
CORRELATIONS
  /VARIABLES=DVA_Start_year1 DVA_Start_Year2 DVA_Year1_Year2 DCMT_Start_Year1 DCMT_Start_Year2
    DCMT_Year1_Year2 BetweenTime_1 BetweenTime_2 TimeBetweenInj
  /PRINT=TWOTAIL NOSIG
  /MISSING=PAIRWISE.
```

Correlations

Notes

Output Created	27-NOV-2016 18:43:50
Comments	

Input	Data	E:\2016_MFK\Konsultasie\Ander\EttienneLeRoux20161121.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	53
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
Syntax		CORRELATIONS /VARIABLES=DVA_Start_year1 DVA_Start_Year2 DVA_Year1_Year2 DCMT_Start_Year1 DCMT_Start_Year2 DCMT_Year1_Year2 BetweenTime_1 BetweenTime_2 TimeBetweenInj /PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.
Resources	Processor Time	00:00:00.03
	Elapsed Time	00:00:00.03

		Change in VA from Start to Year 1	DVA_Start_Year2	DVA_Year1_Year2
Change in VA from Start to Year 1	Pearson Correlation	1	.700**	-.427*
	Sig. (2-tailed)		.000	.037
	N	52	24	24
DVA_Start_Year2	Pearson Correlation	.700**	1	.346
	Sig. (2-tailed)	.000		.097
	N	24	24	24
DVA_Year1_Year2	Pearson Correlation	-.427*	.346	1
	Sig. (2-tailed)	.037	.097	
	N	24	24	24
Change in CMT from start to Year 1	Pearson Correlation	-.164	-.191	.077
	Sig. (2-tailed)	.269	.372	.721
	N	47	24	24
DCMT_Start_Year2	Pearson Correlation	-.233	-.182	.077
	Sig. (2-tailed)	.272	.396	.722
	N	24	24	24
DCMT_Year1_Year2	Pearson Correlation	.001	.007	.007
	Sig. (2-tailed)	.995	.974	.973
	N	24	24	24
BetweenTime_1	Pearson Correlation	.124	.179	-.152
	Sig. (2-tailed)	.381	.403	.479
	N	52	24	24
BetweenTime_2	Pearson Correlation	.056	.100	.030
	Sig. (2-tailed)	.789	.642	.890
	N	25	24	24

TimeBetwee nInj	Pearson Correlation	.071	.174	.011
	Sig. (2-tailed)	.614	.417	.958
	N	52	24	24

		Change in CMT from start to Year 1	DCMT_Start_Year2	DCMT_Year1_Year2
Change in VA from Start to Year 1	Pearson Correlation	-.164	-.233	.001
	Sig. (2-tailed)	.269	.272	.995
	N	47	24	24
DVA_Start_Year2	Pearson Correlation	-.191	-.182	.007
	Sig. (2-tailed)	.372	.396	.974
	N	24	24	24
DVA_Year1_Year2	Pearson Correlation	.077	.077	.007
	Sig. (2-tailed)	.721	.722	.973
	N	24	24	24
Change in CMT from start to Year 1	Pearson Correlation	1	.941**	-.070
	Sig. (2-tailed)		.000	.744
	N	48	24	24
DCMT_Start_Year2	Pearson Correlation	.941**	1	.272
	Sig. (2-tailed)	.000		.199
	N	24	24	24
DCMT_Year1_Year 2	Pearson Correlation	-.070	.272	1
	Sig. (2-tailed)	.744	.199	
	N	24	24	24
BetweenTime_1	Pearson Correlation	.046	-.125	-.035
	Sig. (2-tailed)	.754	.560	.869
	N	48	24	24

BetweenTime_2	Pearson Correlation	-.178	-.208	-.208
	Sig. (2-tailed)	.395	.329	.329
	N	25	24	24
TimeBetweenInj	Pearson Correlation	.043	-.194	-.168
	Sig. (2-tailed)	.770	.365	.432
	N	48	24	24

Correlations

		BetweenTime_1	BetweenTime_2	TimeBetweenInj
Change in VA from Start to Year 1	Pearson Correlation	.124	.056	.071
	Sig. (2-tailed)	.381	.789	.614
	N	52	25	52
DVA_Start_Year2	Pearson Correlation	.179	.100	.174
	Sig. (2-tailed)	.403	.642	.417
	N	24	24	24
DVA_Year1_Year2	Pearson Correlation	-.152	.030	.011
	Sig. (2-tailed)	.479	.890	.958
	N	24	24	24
Change in CMT from start to Year 1	Pearson Correlation	.046	-.178	.043
	Sig. (2-tailed)	.754	.395	.770
	N	48	25	48
DCMT_Start_Year2	Pearson Correlation	-.125	-.208	-.194
	Sig. (2-tailed)	.560	.329	.365
	N	24	24	24
DCMT_Year1_Year2	Pearson Correlation	-.035	-.208	-.168
	Sig. (2-tailed)	.869	.329	.432
	N	24	24	24

BetweenTime_1	Pearson Correlation	1	.660**	.856**
	Sig. (2-tailed)		.000	.000
	N	53	25	53
BetweenTime_2	Pearson Correlation	.660**	1	.913**
	Sig. (2-tailed)	.000		.000
	N	25	25	25
TimeBetweenInj	Pearson Correlation	.856**	.913**	1
	Sig. (2-tailed)	.000	.000	
	N	53	25	53

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Patient Study Number	**Follow-up Period**	**AGE**	**SEX**	**RACE**	**OD/OS**
1	>1y	80	M	I	OS
2	>1y	82	F	W	OD
3	>1y	72	M	W	OD
4	>1y	80	F	W	OD
5	>2y	79	M	I	OD
6	>2y	83	F	W	OD
7	>1y	89	M	W	OD
8	>1y	90	M	W	OS
9	>2y	93	M	W	OD
10	>1y	72	M	W	OD
11	>1y	86	M	W	OS
12	>1y	86	F	W	OD
13	>1y	82	F	I	OS
14	>2y	86	F	W	OD
15	>1y	76	M	W	OD
16	>2y	84	F	I	OS
17	>2y	77	M	W	OS
18	>2y	80	M	W	OD
19	>1y	86	M	W	OD
20	>1y	62	M	W	OD
21	>1y	79	M	W	OD
22	>1y	83	M	W	OD
23	>2y	83	M	W	OS
24	>2y	83	M	W	OD
25	>2Y	86	M	I	OD
26	>2Y	86	M	I	OD
27	>1Y	72	F	W	OS
28	>2y	84	F	W	OS
29	>1Y	81	M	W	OD
30	>1Y	81	M	W	OS
31	>2y	73	F	B	OS
32	>1y	61	F	W	OD
33	>2y	73	M	W	OS
34	>2y	93	F	B	OD
35	>2y	75	F	W	OS
36	>2y	77	F	W	OS
37	>2y	76	F	W	OD
38	>2y	73	M	W	OD
39	>2y	79	M	I	OD
40	>2y	68	F	W	OS
41	>2y	83	M	W	OD
42	>2y	84	F	W	OD
43	>2y	84	F	W	OS
44	>1y	76	F	W	OS
45	>1y	68	M	W	OS
46	>1y	86	M	W	OD

47	>1y	75	F	W	OD
48	>1y	75	F	W	OS
49	>1y	71	F	W	OD
50	>1y	75	M	W	OS
51	>1y	74	M	I	OD
52	>1y	75	F	W	OS
53	>1y	71	F	B	OS
Patient Study Number	**Exclusion Reason**	**AGE**	**SEX**	**RACE**	**OD/OS**
53 eyes of 48 patients	3 eyes with 1y follow-up		29 Male	3 African	OS-Left-22
	24 eyes with 2y follow-up		24 Female	8 Indian	OD-Right-31
				42 Caucasian	
		Counting Fingers vision given value of 0			
		1.0 is 6/6 vision the closer to 1.0 the bet			

VA Sart	CMT Start	Date IVI-1	VA 1 year	CMT 1 year	VA 2 year
0.1	268	11/03/2015	0.15	234	
0.1	472	10/07/2013	0.05	774	
0.4	215	01/07/2015	0.7	185	
0.1	275	22/06/2011	0.15	134	
0.3	348	08/06/2011	0.2	299	0.3
0.1	501	15/05/2013	0.2	358	0.2
0.3	258	05/10/2011	0.3	223	
0.1	277	27/06/2012	0.1	260	
0.3	329	10/08/2011	0.3	216	0.3
0.09	316	12/09/2012	0.1	264	
0.3	305	13/05/2015	0.6	257	
0.05	335	16/05/2012	0.05	265	
0.3		11/01/2012	0.5		
0.3	233	21/09/2011	0.5	209	0.3
0.15	346	20/04/2011	0.1	310	
0.15	234	16/11/2011	1.0	187	0.4
0.5	297	20/04/2011	0.5	270	0.6
0.2	351	20/04/2011	0.6	247	0.1
0.2	284	22/06/2011	0.2	245	
0.3		24/06/2015	0.3		
0.3		16/08/2011	0.5		
0.15	324	30/01/2013	0.05	207	
0.15	379	05/10/2011	0.3	314	0.2
0.1	370	25/01/2012	1.0	177	1.0
0.05	340	27/6/2012	0.15	285	0.6
0.15	457	04/04/2012	0.5	296	0.3
0.01	392	26/11/2014	0.5	266	
0.3	225	30/05/2012	0.15	205	0.15
0.2	239	08/08/2012	0.5	233	
0.15	611	15/08/2012	0.15	253	
0.1	274	03/10/2012	0.15	264	0.1
0.2	373	24/06/2015	0.3	355	
0.3	321	21/11/2012	0.8	292	0.8
0.15	232	05/12/2012	0.15	175	0.3
0.15	342	28/11/2012	0.5	327	0.8
0.5	307	30/01/2013	0.4	312	0.4
0.2	294	30/01/2013	0.4	220	0.2
0.5	242	29/05/2013	0.2	233	0.7
0.1	509	17/07/2013	0.3	274	0.4
0.15	298	19/02/2014	0.6	262	0.8
0.1	348	29/01/2014	0.5	187	0.7
0.8	245	27/08/2014	1.0	222	0.8
0.8	335	05/03/2014	0.2	209	0.3
0.4		23/03/2015	0.7		
0.1	508	29/10/2014	0.2	388	
0.4	420	19/11/2014	0.9	184	

0.2	286	03/12/2104	0.5	230	
0.01	364	10/12/2014	0.4	274	
0.05	344	11/02/2015	0.15	239	
0.05	264	11/02/2015	0.9	205	
0.05	305	01/04/2015	0.4	333	
0.05	414	20/05/2015	0.15	379	
0.01		03/06/2015	0.1		
VA Sart	CMT Start	Date IVI-1	VA 1 year	CMT 1 year	VA 2 year
uity - Vision	Macular Thickness		VA Year 1	CMT	VA Year 2
tart 53 Eyes	Start - 48		53	Year 1 - 48	24
.01 or 6/600	n Micrometers (um)				
ter the vision					

CMT 2 year	Date IVI-2	Date IVI-3
	(weeks since previous injection)4	4
	4	4
	4	5
	(First Highlighted value is the last Injection of the first year) 4	5
265	5	4
332	4	4
	4	4
	4	4
220	(Second highlighted value is the last injection of year 2) 4	4
	4	4
	4	4
	4	4
	4	4
238	4	4
	4	4
188	4	4
258	9	4
249	9	4
	4	3
	4	4
	5	4
	4	4
276	4	4
172	4	5
267	4	4
270	4	7
	4	4
232	4	6
	4	4
	4	4
200	4	4
	4	4
270	4	4
173	5	4
349	4	4
334	4	4
205	4	4
231	4	4
306	4	4
247	4	4
189	4	4
224	5	4
204	6	4
	4	4
	4	3
	4	4

	4	4
	4	4
	4	4
	4	4
	4	4
	4	4
	4	4
CMT 2 year	Date IVI-2	Date IVI-3
CMT		
Year 2 - 24		

Date IVI-4	Date IVI-5	Date IVI-6	Date IVI-7	Date IVI-8	Date IVI-9
4	4	4	4	4	5
4	5	9	4	4	4
6	22	**7**			
4					
16	4	4	6	6	**6**
4	4	4	4	4	6
4	6	8	8	8	**10**
4	5	6	8	8	**10**
8	4	6	8	8	**8**
4	5	6	6	8	4
4	5	5	7	6	12
4	5	7	13	5	**6**
4	4	6	**8**		
4	4	6	8	10	**6**
10	7	12	4	4	**8**
4	4	6	8	4	4
5	4	4	6	8	**10**
4	4	4	4	4	4
4	**26**				
4	4	4	6	6	4
8	4	8	4	4	4
4	14	**4**			
4	4	4	6	8	8
3	6	6	8	**9**	9
4	6	6	8	10	4
4	4	5	6	6	**8**
4	4	4	4	9	5
4	6	8	10	**4**	6
4	6	5	7	8	5
4	4	5	6	8	**12**
6	4	4	4	4	4
4	4	4	6	4	6
6	8	10	**12**	12	14
6	8	10	**12**	**14**	
10	4	4	4	4	4
6	4	4	4	4	4
4	4	4	4	4	7
4	5	6	8	10	**10**
4	4	5	7	9	4
4	4	4	4	4	4
4	4	4	5	4	5
6	7	8	10	**12**	12
6	8	9	6	**7**	8
6	8	8	**10**		
4	4	4	4	4	4
6	8	12	8	**10**	

4	6	4	6	4	4
5	6	6	4	4	4
4	4	6	8	6	6
4	5	6	7	**7**	
4	4	4	4	5	4
4	5	6	6	6	5
4	4	4	4	8	6
Date IVI-4	Date IVI-5	Date IVI-6	Date IVI-7	Date IVI-8	Date IVI-9

Date IVI-10	Date IVI-11	Date IVI-12	Date IVI-13	Date IVI-14	Date IVI-15
4	4	**4**			
4	4	**4**			
4	6	7	8	8	4
6	**8**	6	6	6	6
10	10	12	**14**		
4					
4					
8	9	11	13	**12**	
5	7	7	9	4	6
10	4	4	4	5	4
4	**8**	10	10	**11**	
5	4	**6**			
4	4	**4**			
8	9	12	14	**4**	
12	**12**				
6	8	4	6	4	6
10	4	6	8	4	6
4	4	**4**			
6	8	8	12	6	**8**
6					
5	4	4	**4**	4	4
6	4	**4**			
16	**16**				
4	5	**6**	8	8	10
5	**4**	12	4	6	6
4	6	**6**	8	10	11
12	**14**				
6	8	4	4	4	4
4	4	6	**5**	5	4
4	5	4	**4**	5	6
12	**12**				
10	12	12	**12**		
4	4	4	**4**		

6	**8**				
4	**8**				
7					
4	**11**				
7	**6**				
4					
Date IVI-10	Date IVI-11	Date IVI-12	Date IVI-13	Date IVI-14	Date IVI-15

Date IVI-16	Date IVI-17	Date IVI-18	Date IVI-19	Date IVI-20
4	6	4	**6**	
13	8	4	**4**	
6	8	**4**		
9	4	**6**		
8	4	4	4	**4**
4	6	8	**4**	
14	4	4	4	4
11	**11**			
8	10	**9**		
12	**12**			
6	6	7	**7**	
4	5	6	6	5
7	7	**9**		

Date IVI-16	Date IVI-17	Date IVI-18	Date IVI-19	Date IVI-20

Date IVI-21	Date IVI-22	Total number Injection Year 1
		12
		12
		6
		4
		9
		11
		9
		9
		9
		10
		10
		9
		7
		9
		9
		10
		9
		11
		4
		12
		12
		6
		10
		8
		10
		10
		12
		8
		10
		9
4	**4**	13
		12
		7
		7
		12
		11
		12
		9
		10
4	**6**	13
		13
		8
		8
		7
		13
		8

		11
		11
		10
		8
		11
		11
		10
Date IVI-21	Date IVI-22	Total number Injection Year 1
		Total = 511
		Mean = 9.64

Total number Injections Year 2				
10				
8				
4				
5				
8				
9				
3				
4				
3				
10				
9				
7				
9				
4				
1				
5				
7				
5				
2				
9				
9				
5				
3				
5				

Total number Injections Year 2				
Total = 144				
Mean = 6				