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Rehabilitation Leprosy control AIDS prevention Drug education

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Statistics & Research Department

29 April, 2003

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मिति 1 May 2003

The Chairman,
Nepal Health Research Council
Ramshah Path,
Kathmandu

Sub: **Final report of Cyclosporin A study in leprosy type I reaction.**

Dear Sir,

Please find the final report of the study carried out at Green Pastures Hospital "**Cyclosporin A as Immunosuppresive therapy in leprosy use in type I reaction and pharmacokinetic study in Nepali population**". This study was carried out in conjunction with London School of Hygiene and Tropical Medicine, University of London sponsored by LEPRA [British Leprosy Relief Association]. As a responsible institution for this study, we have the pleasure to submit this report on be half of Dr. Ang Tshering Lama Sherpa, the Principal Investigator, who is out of country at present.

Please don't get hesitate to get back to us if you have any questions regarding the study. Thank you for the good cooperation and hope the same in future.

With kind regards,

Himalaya Dev Sigdel
Statistics & Research Department Manager

London School of Hygiene & Tropical Medicine
(University of London)
Department of Infectious and Tropical Diseases
Keppel Street, London WC1E 7HT

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contributing to health worldwide

INFIR 2

FINAL REPORT

On Trials of alternative treatments for leprosy Type 1 Reactions

Submitted to: **ALERT**
Ethiopian Institute of Science and Technology
Fondation Luxembourgeoise Raol Follereau- Luxembourg
International Nepal Fellowship
LEPRA
London School of Hygiene & Tropical Medicine
Nepal Health Research Council
The Leprosy Mission

Prepared by: **Dr Sharon Marlowe**
Dr Diana Lockwood

Date: **8th January 2003**

Preliminary material

Contents

Abbreviations

ALERT	----------	The All Africa Leprosy & Rehabilitation Training Centre
CyA	----------	Cyclosporin A
GPH	----------	Green Pastures Hospital
INF	----------	International Nepal Fellowship
LSHTM	----------	London School of Hygiene & Tropical Medicine
NHRC	----------	Nepal Health Research Council
TLM	----------	The Leprosy Mission
WBA	----------	Whole blood assay

Acknowledgements

I am grateful to all the doctors, nurses and paramedical workers that took part in these studies and for all their hard work in ensuring that this project could be finished.
I also acknowledge Folleraux Luxembourg, LEPRA and Panacea Biotec (Indian Drug company that provided Panimune, generic CyA) for their generous support. I would like to thank committees at ALERT, Anandaban, the Ethiopian Institute of Science & Technology, GPH , INF, LSHTM, , NHRC, and TLM for allowing this work to be carried out.

Summary and principal action points

The INFIR 2 trials were a group of 3 trials looking into the possibility of using alternative drugs to prednisolone in the treatment of leprosy Type 1 Reactions (T1R). The first trial was to assess whether the combination of azathioprine with a shorter 8 week- course of prednisolone would be as good as the 12 week course recommended by the WHO. This trial was to assess the use of azathioprine as a steroid-sparing agent for severe T1R. The second and third trials were to assess the use of an Indian generic formulation of Cyclosporin A (CyA) as monotherapy for severe leprosy T1R in Nepal and Ethiopia respectively. All 3 trials of INFIR 2 were funded by Folleraux Luxembourg through LEPRA and started in October 1999, under the co-ordination of Dr Sharon Marlowe at LSHTM. The Principle Investigator was Dr Diana Lockwood at LSHTM.

From October 1999 to April 2000, full protocols were written for all 3 trials and ethical approval received from the committees of the London School of Hygiene & Tropical Medicine (LSHTM), Green Pastures Hospital (GPH) and the Nepal Health Research Council (NHRC). Ethical approval was not received from the Ethiopian national committee until December 2000. A Clinical Severity Score (CSS) was also developed to be used as a quantitative method of assessing severity of clinical disease and to be used as a monitoring tool to assess clinical response to CyA therapy. From April to October 2000, the CSS was trialed at GPH and Anandaban hospital to assess it for ease of use and in comparison with the clinical opinion of senior leprologists.

Recruitment of patients for the Azathioprine trial began in May 2000 and was completed by August 2000. All treatment and clinical follow-up of patients in this trial was completed by the end of January 2002. For the CyA trial in Nepal, patients could potentially have been recruited from Oct 2000 to March 2002, but the last patient that entered the trial was in December 2001, as no more patients that were willing or eligible to enter were seen from January - March 2002. Recruitment for the CyA trial in Ethiopia was from July 2001 to March 2002. The last patients from Ethiopia completed their treatment and follow-up assessments at the end of September 2002. For the purposes of this report an interim analysis of the first 14 patients (total 33) from Ethiopia will be presented.

The main outcomes of INFIR 2 are:

- Azathioprine is a safe drug that can be used on an out-patient basis in severe leprosy T1R patients and appears to be effective as a steroid-sparing agent in treating these patients.
- A randomised controlled trial needs to be carried out assessing the combination of short course prednisolone plus a longer course of azathioprine against a longer course of prednisolone.
- Panimune, the Indian generic formulation of CyA appears to be safe and useful as monotherapy in the treatment of severe leprosy T1R in Nepal and Ethiopia.
- Ethiopian T1R patients may require a higher CyA dose than Nepalis due to genetic differences in the way that they absorb CyA.
- A double blind randomised controlled trial is required to compare the efficacy of Panimune with prednisolone.

Introduction and background

Leprosy is complicated by immunological phenomena called reactions. Reactions describe the sudden appearance of symptoms and signs of acute inflammation and occur in approximately 30% of leprosy patients. This occurs due to immune reactions against antigenic components liberated from the bacilli. Patients can present in reaction prior to antibacterial multi-drug treatment (MDT) and a significant proportion of patients will develop reactions within the first six months of treatment. There is also an increase in the incidence of reactions in post-partum patients. However reactions can occur after successful MDT treatment and are due to persistence of the *M. leprae* antigen. (Whitty & Lockwood, 1999)

The management of reactions is currently the most challenging problem. Type 1 or Reversal Reactions (T1R) involve local skin and nerve inflammation and peripheral nerve function impairment. This nerve function impairment is currently the most disabling feature of the leprosy disease with some patients suffering from recurrent or repeated reactions. The only treatment available for severe T1R is oral prednisolone, however 30 – 40% of patients may not respond to this treatment or may become steroid-dependent, with a reduction or cessation of prednisolone causing a flare of symptoms.

Azathioprine

Azathioprine (Azathioprine) is an immunosuppressant that has been used in other immune-mediated diseases both as monotherapy and in combination with prednisolone as a steroid-sparing agent. Azathioprine was first synthesised in 1957 and was used initially to prolong survival of renal allografts (Pearson et al, 1995). It is now a well-established drug in the prevention of human-organ transplant rejection as well as an immunosuppressant in inflammatory bowel diseases (IBD), rheumatoid arthritis and psoriasis. It is known to inhibit the proliferation of lymphoid cells, mainly affecting T-cell mediated reactions. We hypothesised that by combining azathioprine with prednisolone we could reduce the overall dose and course of prednisolone that was needed for severe T1R.

Cyclosporin A

Cyclosporin A (CyA) selectively inhibits the activation of CD4 T cells and the expression of cytokines such as IL-2. Available evidence also suggests that CyA acts reversibly on T cells. It is currently widely in use in diseases such as psoriasis, rheumatoid arthritis and for human transplant immunosuppression. We hypothesised that CyA could switch off the immune response that was responsible for T1R, since we know that T1R is due to an increased T cell mediated immune response.

Assignment terms of reference

The aims of these 3 trials were:

For the Azathioprine trial comparing short course prednisolone plus azathioprine (AP) with longer course prednisolone (P) at Anandaban Hospital

(1) assess whether the amount of extra prednisolone differed in the 2 treatment groups
(2) assess whether the clinical parameters of T1R were differentially influenced by the drugs used
(3) assess whether cytokines detected in skin and whole blood were differentially influenced by the drugs used
(4) assess whether clinical disease activity correlated with amounts and types of cytokines detected
(5) assess the safety of using azathioprine for Nepali patients with severe T1R

For the CyA trial assessing the clinical response of severe T1R patients in Nepal and Ethiopia to an Indian generic formulation of CyA (Panimune)

(1) assess the effectiveness of Panimune as monotherapy for severe CyA.
(2) determine whether there is a different response to CyA in Nepali and Ethiopian patients with T1R
(3) assess the safety of CyA in Nepali and Ethiopian patients with T1R.

Assignment report

Strategy

For the Azathioprine trial at Anandaban Hospital –Nepal

This was an open prospective pilot study comparing 19 patients on the WHO –recommended 12 week course of prednisolone (P) with 21 patients on an 8 week course of prednisolone plus 12 weeks of azathioprine

TREATMENT REGIMEN

40 PATIENTS

21 AP patients on AZATHIOPRINE 3mg /kg/day for 3/12 Plus Pred:	19 P patients on PRED alone:
40 mg – 2/52	40 mg – 2/52
30 mg – 2/52	30 mg – 2/52
20 mg – 1/52	20 mg - 2/52
15 mg - 1/52	15 mg – 2/52
10 mg – 1/52	10 mg – 2/52
5 mg - 1/52	5 mg – 2/52

Total prednisolone dose in the AP arm – 190 mg over 8 weeks
Total prednisolone dose in the P arm - 240 mg over 12 weeks

If patients did not improve in either the AP or P arm the dose of prednisolone could be increased according to predetermined criteria.

CYTOKINE ANALYSIS

A whole blood assay was used to detect cytokine production in each patient before, during and after treatment. Cytokines assessed were tumour necrosis factor-α, (TNF-α) interleukin-10 (IL-10) and gamma interferon (IFN-γ) at day 0 and weeks 2, 8, 12 and 16. Whole blood was stimulated with the following antigens in an overnight assay: *M. leprae* sonicate (MLS), whole *M. leprae* (WML), *M. leprae* cytosolic fraction (MLSA-LAM) and cell wall fraction (MLCwA). Supernatants were harvested and cytokine detection was carried out using an ELISA method.

Cytokine production in the skin was assessed for each patient at two time points, baseline and during or at the end of treatment. Monoclonal antibodies directed against TNF-a, IL-10 and IL-2 were used with immunostaining to semi-quantitatively visualize the amount of cytokine present in the skin section.

CyA trials at GPH and ALERT

These were open prospective pilot studies with the aim to recruit 40 Nepali T1R patients at GPH and 40 Ethiopian T1R patients at ALERT.

TREATMENT REGIMEN

All patients were treated with 12 weeks CYA at a starting dose of 5 mg/kg/day. For the first 5 days 40mg prednisolone was added to the CyA since CyA takes a few days to have a therapeutic effect. The study protocols and predetermined criteria allowed for the dose of CyA could be increased up to 7.5 mg/kg/day if there was no clinical improvement or clinical deterioration was seen. If patients did not respond to this increased dose then they were taken out of the trial. Doses of CyA could also be reduced according to study protocols if any side effects were seen.

Observations

Prior to this study, CyA was not a drug that was licensed for general use in Nepal. Therefore, we had to obtain clearance from the Nepali Drug Administration Authorities to import these drugs into Nepal from India. The drugs reached GPH by October 2000.

Everything was in place to start recruitment at GPH by October 2000, but due to the harvest season in Nepal, the first patient was not recruited until December 2000. 10 patients had been recruited at GPH from December 2000 to March 2002. The reasons for the low number of patients being recruited are:

- Only patients with severe Type 1 reaction (T1R) were recruited into this study. This was because CyA was to be studied as a second-line treatment for severe T1R only, since there is adequate treatment for mild and moderate reactions, but no treatment for severe reaction cases.
- During the recruitment period, there were mounting political problems in Nepal in addition to the massacre of the Nepali Royal Family. This affected recruitment since many patients had to travel long distances to get to GPH and due to curfews and national shut-downs the patient attendance at the hospital dropped off significantly.

A pleasing outcome was the increased confidence that clinicians at the different sites developed in using the immunosuppressants. Initially there was concern that use of these immunosuppressants could lead to unwanted side effects. Currently all clinicians involved are happy with the response to both the azathioprine and CyA and are enthusiastic to both publicise these results and take part in the next (hopeful) phase of multi-centre blinded controlled trials assessing the true efficacy of these drugs with a larger sample size. There was even a change in policy during the study allowing out-patients as well as in-patients to take part in the azathioprine trial at Anandaban.

In these studies, albeit with small numbers of patients, with careful monitoring the incidence of side-effects is low.

Analysis

Summary of azathioprine clinical results

- the AP and P treatment produce similar clinical outcomes
- skin signs: 52% of AP and 63% of P patients improved
- acute sensory function impairment (less than or equal to 6 months duration prior to presentation): 63% of the AP and 50% of the P patients improved their sensory function
- acute muscle power impairment (less than or equal to 6 months duration prior to presentation): 50% AP and 63% P patients improved their muscle power
- indications for extra prednisolone:
    - Worsening of skin signs: 43% of AP patients and 37% of P patients
    - Worsening of sensory function: 24% of AP and 16% of P patients
    - Worsening of muscle power: 14% of AP and 11% of P patients
- absence of major side effects with both the AP and P regimens

Summary of azathioprine laboratory results

- Cytokines produced in whole blood: no significant difference between the AP and P groups
- TNF–α production: AP and P groups both showed a significant reduction in TNF-a production in the whole blood assay by week 2, but this reduction disappeared as the dose of prednisolone was reduced
- Cytokines detected in skin: no significant difference between the AP and P groups
- IL12 detected in skin: in the AP group this was significantly reduced by week 12 compared to baseline
- a 12 week course of prednisolone had no significant effect on cytokine expression in the blood or skin
- no correlation was found between baseline TNF-α and IFN-γ detected in skin or blood and clinical outcome
- IL-10 levels produced at baseline were higher in those that improved clinically

Summary of CyA results

- CyA monotherapy could be an effective treatment for severe T1R as a second-line therapy to prednisolone
- Nepalis need a dose of about 5mg/kg/day for a good clinical response, most patients will maintain their response up to 3 months after stopping CyA treatment
- 30% of Ethiopians with severe T1R need a higher dose of CyA (~ 7.5mg/kg/day) to have a good clinical response to CyA monotherapy.
- 75% of Ethiopians who responded to CyA monotherapy, even on the higher dose did not maintain this response once treatment was stopped
- Genetic studies of both the Nepali and Ethiopian patients may explain these differences. Ethiopians have a particular genotype that is associated with lower intestinal absorption of CyA and therefore need a higher dose of CyA to reach the same blood level of CyA as the Nepalis.

Conclusions

We conclude from these preliminary studies that:

- the combination of azathioprine with prednisolone appears to allow a shorter course of prednisolone to be used with no worsening of outcome
- a 12 week course of prednisolone dose not seem adequate to control symptoms of severe Type 1 reactions since more than a third of patients on this regimen required extra prednisolone to control their reaction symptoms
- a larger scale double blind randomised controlled trial is needed out to compare the efficacy of azathioprine as a steroid-sparing agent in the use of severe Type 1 reactions
- the Indian generic formulation of Cyclosporin A , Panimune may have a role in treatment of patients who fail to respond to prednisolone or in whom prednisolone is contraindicated
- Ethiopian patients can tolerate a higher dose of panimune than Nepalis without detrimental effects
- the difference between the Ethiopian and Nepali response to Panimune may be explained by differences in their genotypes in respect of intestinal absorption of drug.
- a larger scale randomised controlled trial comparing panimune with prednisolone needs to be carried out following on from these small studies

Action Points Arising

- The data from these studies is still in the process of being fully analysed and some of the data from Ethiopia is still outstanding
- Once I have completed this analysis, I shall be writing up the results in the form of papers to be published in peer-reviewed journals
- Copies of these papers will be circulated to all persons concerned in this project

Appendix I
Clinical Severity Score

Category	Criteria	Score = 0	Score = 1	Score = 2	Score = 3
A1	Degree of inflammation of skin lesions	None	Erythema	Erythema and raised	Ulceration
A2	% of raised and inflamed skin lesions	<25%	25 – 50%	50 – 75%	>75%
A3	Peripheral Oedema (due to reaction)	None	Minimal	Visible but not affecting function	Oedema affecting function
A4	Nerve pain	None	Pain on activity	Pain at rest	Pain disturbing sleep
A5	Nerve tenderness (worst affected nerve only)	None	Mild Tenderness	Withdrawal or wincing	Not allowing palpation
A6	Fever due to reaction	<37.5°C	37.5 – 38.5°C	38.5 – 40°C	>40°C
B1	R Trigeminal	Felt	XXXXXXX	XXXXXXX	Not Felt
B2	L Trigeminal	Felt	XXXXXXX	XXXXXXX	Not Felt
B3	R Ulnar	All sites felt	1 site not felt	2 sites not felt	3 sites not felt
B4	L Ulnar	All sites felt	1 site not felt	2 sites not felt	3 sites not felt
B5	R Median	All sites felt	1 site not felt	2 sites not felt	3 sites not felt
B6	L Median	All sites felt	1 site not felt	2 sites not felt	3 sites not felt
B7	R Post. Tibial	All sites felt	1 site not felt	2 sites not felt	>=3 sites not felt
B8	L Post Tibial	All sites felt	1 site not felt	2 sites not felt	>=3 sites not felt
C1	R Facial	MRC = 5	MRC = 4	MRC = 3	MRC<3
C2	L Facial	MRC = 5	MRC = 4	MRC = 3	MRC<3
C3	R Ulnar	MRC = 5	MRC = 4	MRC = 3	MRC<3
C4	L Ulnar	MRC = 5	MRC = 4	MRC = 3	MRC<3
C5	R Median	MRC = 5	MRC = 4	MRC = 3	MRC<3
C6	L Median	MRC = 5	MRC = 4	MRC = 3	MRC<3
C7	R Radial	MRC = 5	MRC = 4	MRC = 3	MRC<3
C8	L Radial	MRC = 5	MRC = 4	MRC = 3	MRC<3
C9	R Lat. Popliteal	MRC = 5	MRC = 4	MRC = 3	MRC<3
C10	L Lat. Popliteal	MRC = 5	MRC = 4	MRC = 3	MRC<3

Category B – Sensory Testing (ST). For ulnar and median nerves a 2g monofilament was used to test 3 sites for each nerve in the hand. For Posterior Tibial a 10g monofilament was used to test 4 sites on the sole of the foot.
Category C – Voluntary Muscle Testing (VMT). MRC – Medical Research Council grading for muscle power

Appendix II
Clinical Azathioprine Results

Patients requiring extra prednisolone and reasons

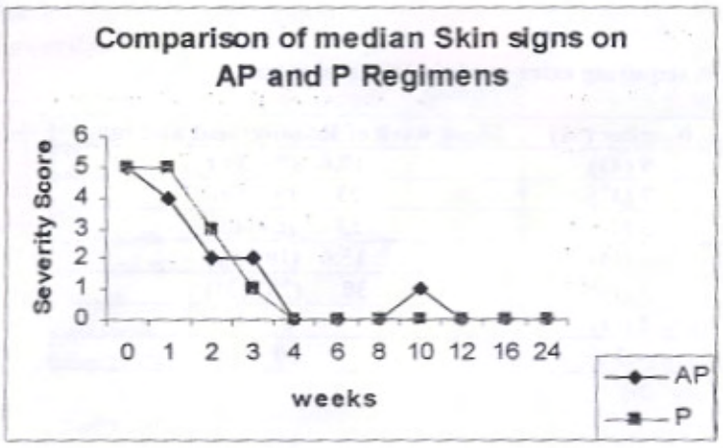
Reason	Regimen	Number (%)	Mean week of Requirement and range
Worsening SS	AP	9 (43)	17.6 (7 – 34)
Worsening SS	P	7 (37)	23 (9 – 60)
Worsening ST	AP	5 (24)	23 (5 – 40)
Worsening ST	P	3 (16)	15.6 (10 -22)
Worsening VMT	AP	3 (14)	30 (28 – 31)
Worsening VMT	P	2 (11)	3
Worsening NPT	P	1	30

Course of treatment and clinical outcome in patients treated with AP or P

Clinical Outcome	AP	P
No. of patients enrolled	21	19
Skin Signs (SS)		
No.(%) improved	11 (52)	12 (63)
No.(%) worse or no change	4 (19)	1 (5)
No.(%) relapsed	6 (29)	6 (32)
Nerve Pain & Tenderness (NPT)		
Zero initial NPT	16 (76)	12(63)
No.(%) improved	4 (80)	5 (71)
No.(%) worse	1 (20)	2 (29)
Sensory Testing (ST)		
Zero initial ST	5 (24)	10 (53)
Number with < =6/12 history initially	8 (38)	6 (32)
No.(%) improved	5 (63)	3 (50)
No.(%) worse or no change	3 (38)	3 (50)
Number with > 6/12 history initially	8 (38)	3 (16)
No. (%) improved	3 (38)	2 (67)
No. (%) worse or no change	5 (63)	1 (33)
Voluntary Muscle Testing (VMT)		
Zero initial VMT	11 (52)	8 (42)
Number with < =6/12 history initially	6 (29)	8 (42)
No.(%) improved	3 (50)	5 (63)
No.(%) worse or no change	3 (50)	3 (38)
Number with > 6/12 history initially	4 (19)	3 (16)
No.(%) improved	1 (25)	1 (33)
No.(%) worse or no change	3 (75)	2 (67)

Comparison of median clinical parameters on AP and P regimens

a)



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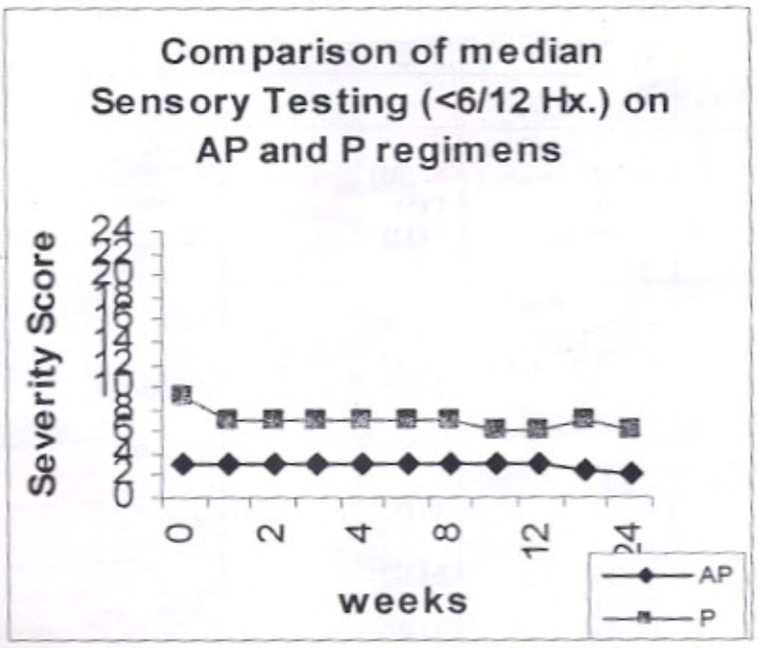
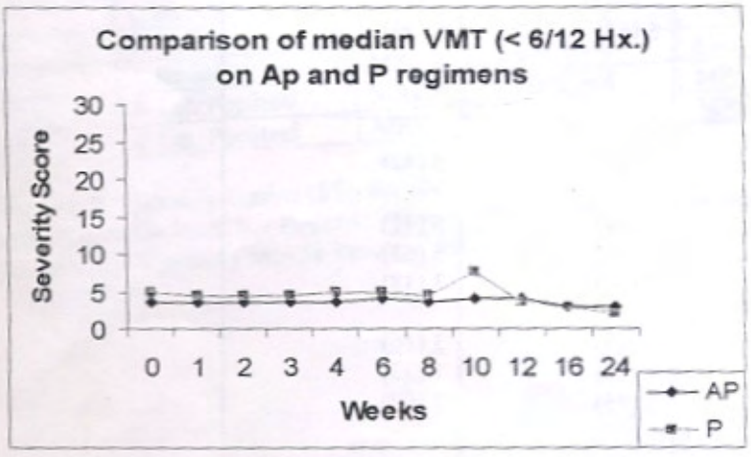


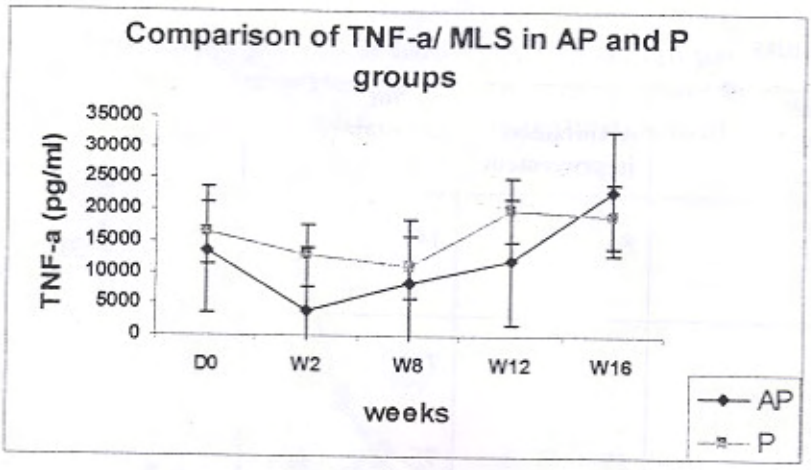
c)



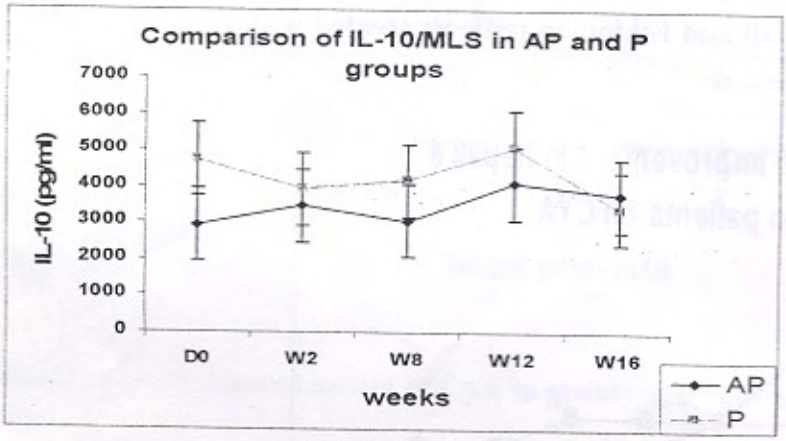
Appendix III

Laboratory Results from Azathioprine study

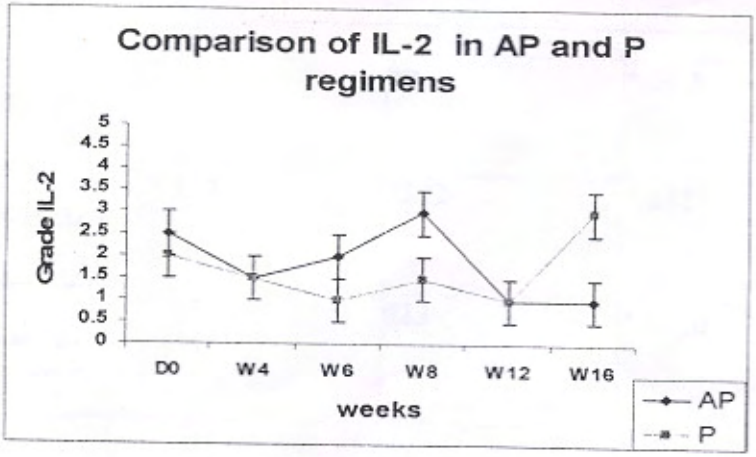
Effect of AP and P on TNF-α produced in the WBA



Effect of AP and P on IL-10 produced in the WBA



Effect of AP and P on IL-2 detected in the skin



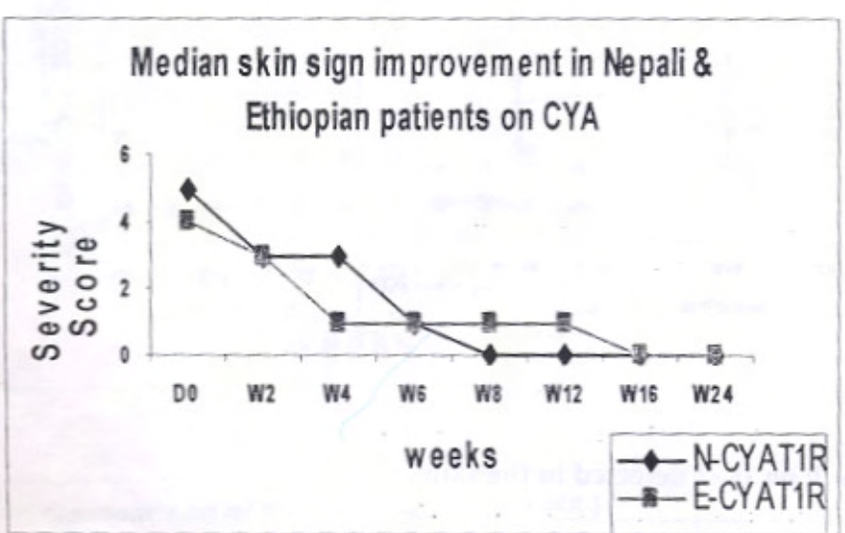
Appendix IV

Clinical Cyclosporin A Results

Clinical CyA Results

	% Improved	Mean dose (mg/kg/day)	% Maintained improvement	% Not maintained
Nepalis (n = 7)	100	4.85	86	14
Ethiopians (n = 13)	62	4.99	25	75
	31	7.59	25	75

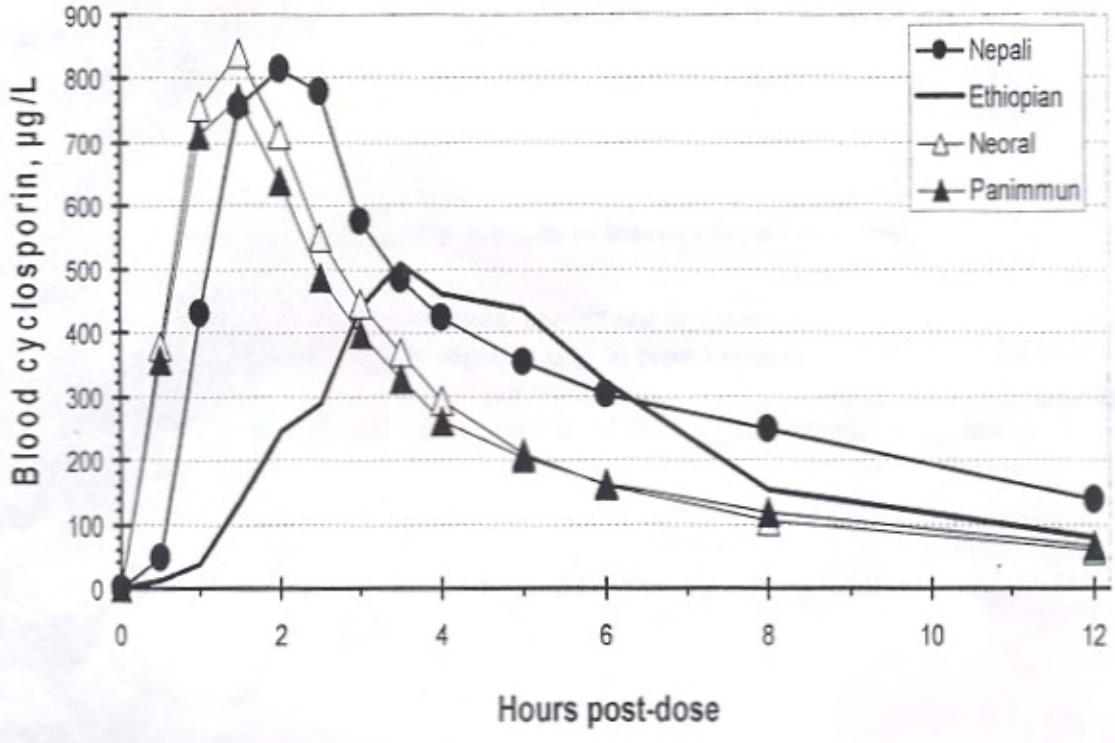
Results of skin signs in the Nepali and Ethiopian patients treated with CyA



Appendix V

Graphs / tables of Pharmacokinetic results of panimune and genotype frequencies in the 2 patient populations

Pharmacokinetic profiles of Ethiopian and Nepali patients on the Indian generic CyA, Panimune compared to Healthy Indian volunteers on Panimune and the "gold standard" preparation, Neoral



Relationship of Pharmacokinetics of CyA to genotype frequency in Ethiopian and Nepali patients

	% CC genotype	% CT genotype	% TT genotype
ALERT (Ethiopian)	* 67	33	* 0
GPH (Nepali)	* 12.5	50	* 37.5
AUC *(Area under the curve)*	* 2395	3222	* 6325
Cmax *(Maximum CyA concentration in blood)*	* 637	823	* 1160