Progress Report for R&D Projects
[Final report, 2015]

Section-A: Project Details

A1. Project Title:

Longitudinal analysis of changes in cytokine profiles of malaria patients at different stages of treatment and disease resolution: Understanding the molecular basis of cure and malarial pathology.

A2. DBT Sanction Order No. & Date: No. BT/CP/11/NE/TBP/2010 dated 28.03.11

A3. Name of Principal Investigator: Dr. Shashi Baruah, Tezpur University, Tezpur
Name of Co-PI/Co-Investigator: Dr. Santosh K.Kar & Dr. Sanjeeb Kakati

A4. Institutes: Tezpur University, Assam Medical College and Hospital & Kalinga Institute of Industrial Technology, Bhubaneshwar

A5. Address with Contact Nos. (Landline & Mobile) & Email:
Department of Molecular Biology and Biotechnology, Tezpur University, Napaam, Tezpur, Assam. Email: sbaruah@tezu.ernet.in; Ph no: +919435082587

A6. Total Cost: 66.66 lakhs

A7. Duration: 3 years

A8. Approved Objectives of the Project:

1) To study the expression of cytokine genes and cytokine protein levels in relation to symptomology of *P.falciparum* malaria

2) To study the temporal changes in the levels of the relevant cytokines with disease progression or regression following drug treatment.

3) To compare the expression of Foxp3 gene in complicated and uncomplicated malaria cases

A9.Specific Recommendations made by the Task Force (if any): NIL

Section-B: Scientific and Technical Progress

B1. Progress made against the Approved Objectives, Targets & Timelines during the Reporting Period

Progress against the Objectives:

Objective 1: To study the expression of cytokine genes and cytokine protein levels in relation to symptomology of *P. falciparum* malaria

Study site and population

The study was conducted in Manja region of Karbi Anglong (ASPR of 7% from Figure 1) district where *Pf* malaria transmission is mesoendemic with intense transmission during the rainy season that usually extends from May to July. Significantly, this was the region where resistance to chloroquine was first detected in Assam, India in 1973. The population of Karbi Anglong is predominantly of Tibeto-Burman linguistic group consisting of ethnic tribes like Karbis, Kukis, Bodos, Dimasas and Garos. Patients recruited from field work included those attending the primary health centers and malaria health camps conducted by National Rural Health Mission (NRHM) as well as from door-to-door visits in remote villages. The severe malaria cases were obtained from Assam Medical College and Hospital, Dibrugarh.

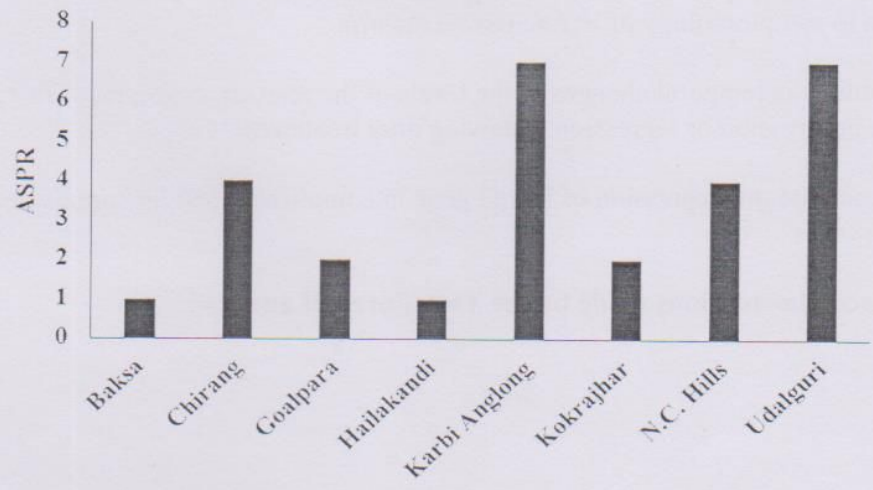


Figure 1: Annual slide positivity rate of year 2011 in districts of Assam, highlighting Karbi Anglong as the selected study site. The data was obtained from Directorate of Health Services, Government of Assam.

Sample collection

Individuals who were diagnosed with *Pf* positivity by pLDH based RDT kits and confirmed by microscopy were recruited in the study. In addition to microscopy, Real-Time PCR approach for quantifying parasite density was adopted. *Pf* 18S rRNA-specific primer and TaqMan probe was used to quantify parasitemia in the clinical samples following the method of Malhotra et al. Hemoglobin levels (g/dl) and glucose levels (mg/dl) were estimated by the use of HemoCue analyzers (HemoCue AB, Angelholm, Sweden). For hospital admitted severe malaria cases, other important clinical investigations were carried out. All the clinical parameters were recorded in a pre-designed questionnaire. Patients admitted in hospital with symptoms of malaria complications as defined by WHO guidelines were grouped under severe malaria (SM). Patients were considered to have uncomplicated malaria (UM) if they had signs and symptoms of malaria without evidence of complications. The healthy status of control participants at the time of recruitment was based on a medical history as well as a physical examination. Non-malarial fever controls (NMF) with symptoms of cold and cough were also recruited in the study.

Peripheral blood in EDTA and RNAlater (Ambion, USA) was obtained from SM (n=51) and UM (n=64) patients by venipuncture on day 0 before treatment i.e. during active infection. For the NMF (n=20) and control group (n=20), only one blood sample was collected. Written, informed consent was obtained from the study participants. The study was approved by the Tezpur University Ethical Committee (DoRD/TUEC/10-14/453 dated 23/09/10).

Clinical and demographic characteristics of malaria patients

Anemia was seen to be the major feature of SM in our study as depicted by low hemoglobin levels (Table 1) as well by the high proportion of SM patients exhibiting this complication (Table 2). SM patients could be differentiated from UM by high parasitemia levels, axillary temperature and neutrophil count.

Table 1: Demographic and clinical characteristics of patients with SM and UM

Characteristics	SM (n=51)	UM (n=64)	p-value
Age (years)	29.7 (8-51)	25.7 (12-52)	0.969
Temperature (°F)	102.2 (100-104)	99.8 (98-102)	**<0.0001**
Hemoglobin (g/dl)	6.7 (3.5-9.4)	9.2 (7.2-13.3)	**<0.0001**
Glucose (mg/dl)	117.8 (23-198)	99.3 (76.9-134)	0.147
Parasitemia (per µl)	24269 (537-57878)	5797 (482-10270)	**0.02**
Platelet count ($x10^3$/µl)	90.4 (30.0-335.0)	91.6 (16.0-160.0)	0.489
Total leukocyte count ($x10^3$/µl)	7.0 (2.6-14.3)	5.5 (4.2-7.1)	0.158
Lymphocyte count ($x10^3$/µl)	1.7 (0.7-2.8)	2.0 (0.9-4.0)	0.494
Neutrophil count ($x10^3$/µl)	4.5 (0.1-12.0)	2.0 (1.0-2.7)	**<0.0001**
Monocyte count ($x10^3$/µl)	0.2 (0.07-0.5)	0.2 (0.08-0.9)	0.913
Eosinophil count ($x10^3$/µl)	0.1 (0.06-0.4)	0.2 (0.05-0.4)	0.112

* Statistical significance determined by Mann-Whitney U test

Table 2: Proportion of severe malaria patients with specific complications

Severe malaria features	Proportion of severe malaria patients
Anemia	40%
Cerebral malaria	27%
Acute renal failure and hepatitis	27%
Thrombocytopenia	6%

Expression of cytokine genes between SM and UM

Blood samples stored in RNAlater were used for total RNA isolation and cDNA generation using RiboPure Blood kit (Ambion) and High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) respectively. Gene expression analysis was then performed using TaqMan based assays (Applied Biosystems) for IL-2, IL-4, TNF-α, IL-1β, IFN-γ, IL-12α, IL-17A, IL-18, IL-8, RANTES, IL-10, IL-13 and TGF-β on StepOnePlus Real-Time PCR System (Applied Biosystems). GAPDH gene was used as the endogenous control for normalization of expression levels while controls were used as the calibrator for mRNA quantification by Comparative C_t method.

Expression levels in UM revealed a balanced cytokine response with increased levels of pro-inflammatory cytokines with a concomitant increase in anti-inflammatory cytokines. In SM,

the expression of TNF-α ($p<0.0001$), IFN-γ ($p<0.0001$), IL-1β ($p<0.0001$) and TGF-β ($p=0.04$) was up regulated compared to UM (Figure 2A and 2C). Interestingly, the comparison showed down regulation in the expression of IL-10 in SM ($p=0.02$) suggesting dysregulated balance of the host immune response (Figure 2C). However, the C_t values of IL-2, IL-4 and IL-13 were beyond the detection limit and these cytokines were not included for further analysis. Modelling of data by logistic regression taking SM and UM as binary variables showed that IL-1β, TNF-α as well as the combination of IFN-γ*IL-18 were predictive for SM with the area under the receiver-operator curve of 0.986 indicating the strength of the model.

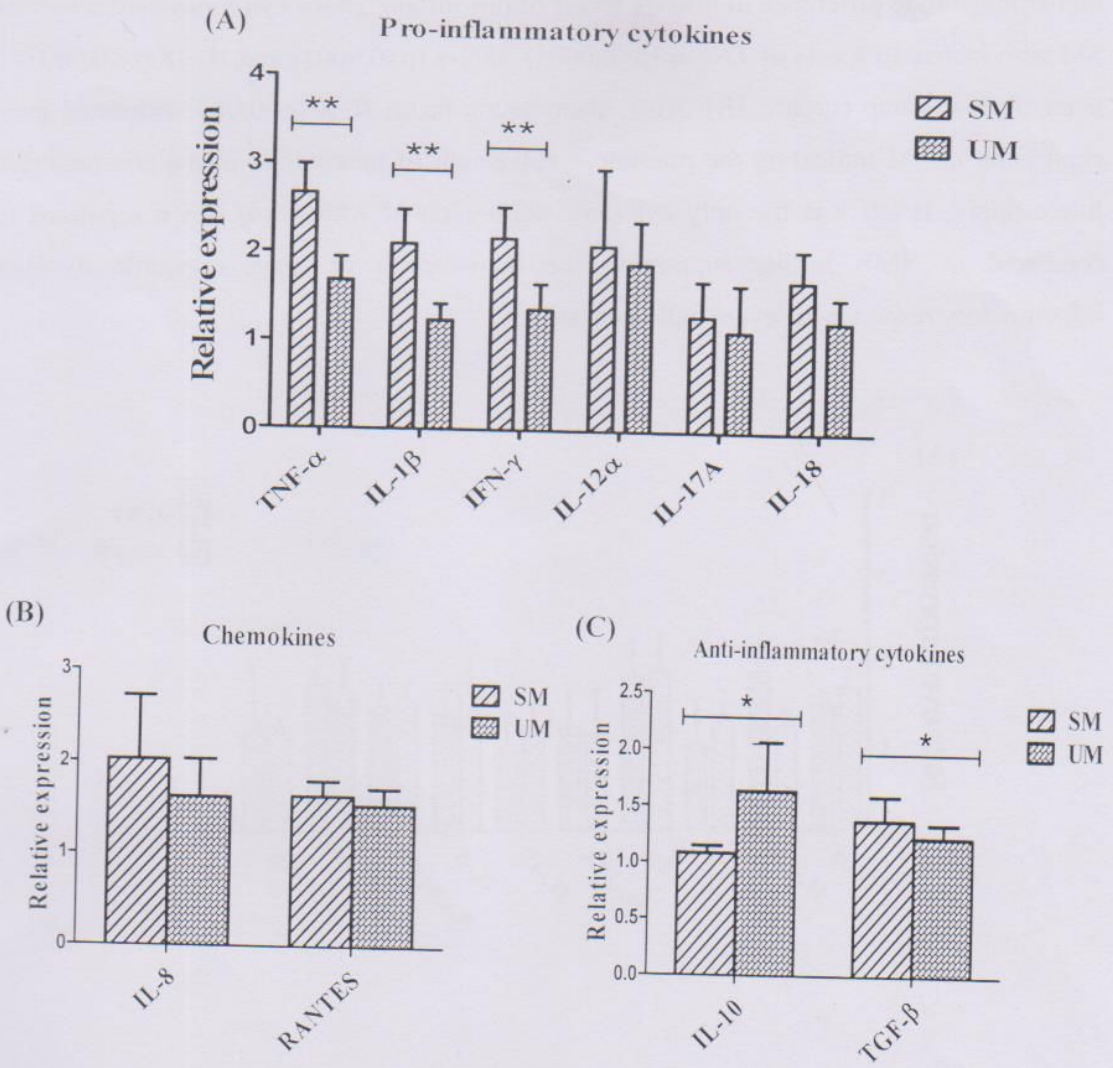
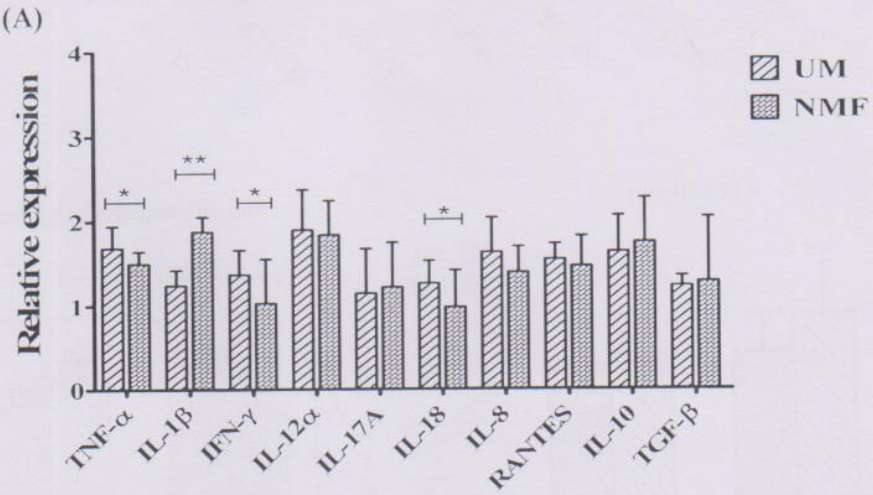


Figure 2: Comparative analysis of mRNA expression profile of (A) Pro-inflammatory cytokines, (B) Chemokines and (C) Anti-inflammatory cytokines between SM and UM on day 0

i.e. during active infection before treatment. Analysis was performed using Unpaired Student's t-test. Single and double asterisks indicate $p<0.05$ and $p<0.0001$ respectively. Error bars represent standard deviation of the mean.

Differential cytokine expression between NMF and malaria clinical status

Modest elevated expression of pro-inflammatory cytokines TNF-α ($p=0.03$), IFN-γ ($p=0.008$) and IL-18 ($p=0.01$) was observed in UM compared to NMF (Figure 3A). In contrast, expression of IL-1β ($p<0.0001$) was highly up regulated in NMF which perhaps could be explained by the fact that IL-1β is a major mediator of fever response. Further, there was a higher magnitude difference in mRNA levels of pro-inflammatory cytokines between NMF and SM with increased levels of TNF-α ($p<0.0001$), IFN-γ ($p<0.0001$) and IL-18 ($p<0.0001$) in the latter disease group (Figure 3B). Also, chemotactic factor IL-8 ($p=0.003$) exhibited increased expression in SM indicating the possible involvement of neutrophils in malaria pathogenesis. Interestingly, IL-10 was the only cytokine, expression of which was down regulated in SM compared to NMF further supporting the involvement of malaria-specific dysregulated inflammatory response in severe pathogenesis.



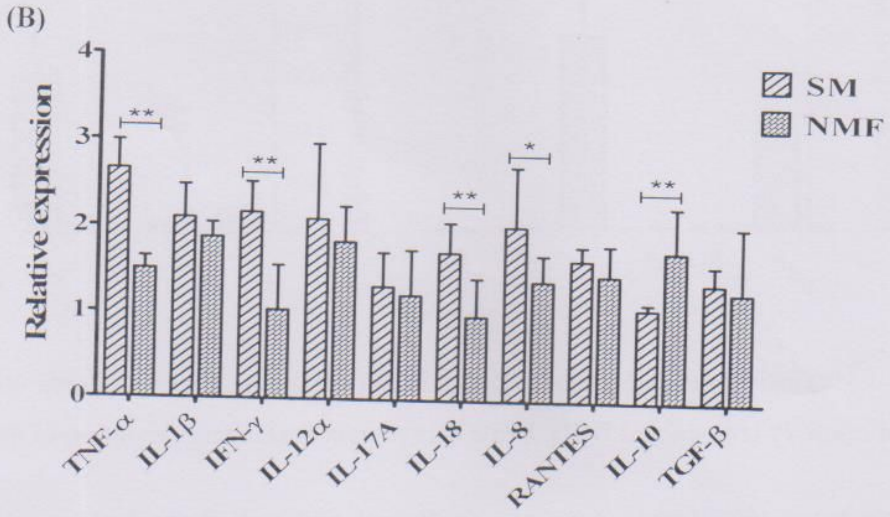


Figure 3: Comparative analysis of mRNA expression profile of cytokines/chemokines between (A) UM and NMF, (B) SM and NMF. Analysis was performed using Unpaired Student's t-test. Single and double asterisks indicate $p<0.05$ and $p<0.0001$ respectively. Error bars represent standard deviation of the mean.

Cytokine protein levels

PBMCs from patients' blood were isolated using Histopaque and adjusted to a final concentration of 10^5 cells/ml in RPMI 1640 culture medium supplemented with 1X Antibiotic-antimycotic solution and 10% Fetal Bovine Serum. Cultures were stimulated with 40µg/ml of crude *Pf* antigen and non-specific mitogen BCG (Bacillus Calmette–Guerin). Crude *Pf* antigen was isolated from in vitro culture of a local *Pf* strain. After 24 hours of incubation, cell-free supernatants were analyzed for cytokine protein levels using the MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel (Millipore, USA) on Millipore's MAGPIX instrumentation platform for selected cytokines while remaining cell pellets were analyzed for mRNA expression levels. The multiplex kit for 96 well plate assay was Human Cytokine/Chemokine Panel I (Cat. No. HCYTOMAG-60K). Absolute concentrations of cytokines were determined from standard curves using Luminex xPONENT software and 5-parameter logistic regression analysis.

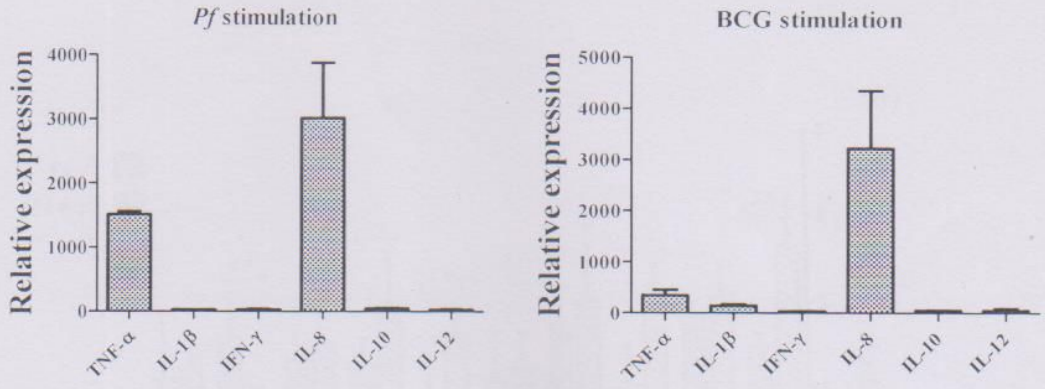


Figure 4: Cytokine/chemokine protein levels in supernatants of cell cultures stimulated with 40μg/ml of crude *Pf* antigen and BCG. Error bars represent standard deviation of the mean.

Correlation between cytokine expression, clinical and parasitological parameters in SM and UM during active infection

In SM, neutrophil counts positively correlated with parasite density and mRNA levels of IL-1β and IL-8 suggesting the role of neutrophils in disease pathology (Figure 5A and 5B). Further, a positive association of parasitemia with TNF-α, IL-1β and IFN-γ was observed (Figure 5C). Interestingly, parasitemia inversely correlated with IL-10 mRNA levels (Figure 5D). Correlation analysis among the cytokines revealed positive association between IFN-γ and TNF-α expression while IL-10 levels were negatively associated with TNF-α, IL-1β, IFN-γ and IL-8 indicating the overproduction of pro-inflammatory cytokines in presence of low IL-10 levels (Figure 5E and 5F). In UM, positive association of parasite count was seen with TNF-α, IL-1β and IL-8. Further, IL-1β positively correlated with TNF-α and IFN-γ. In addition, TGF-β was associated with TNF-α, IL-1β and IFN-γ while IL-10 with TNF-α, IL-1β and TGF-β suggesting a balanced inflammatory response in UM.

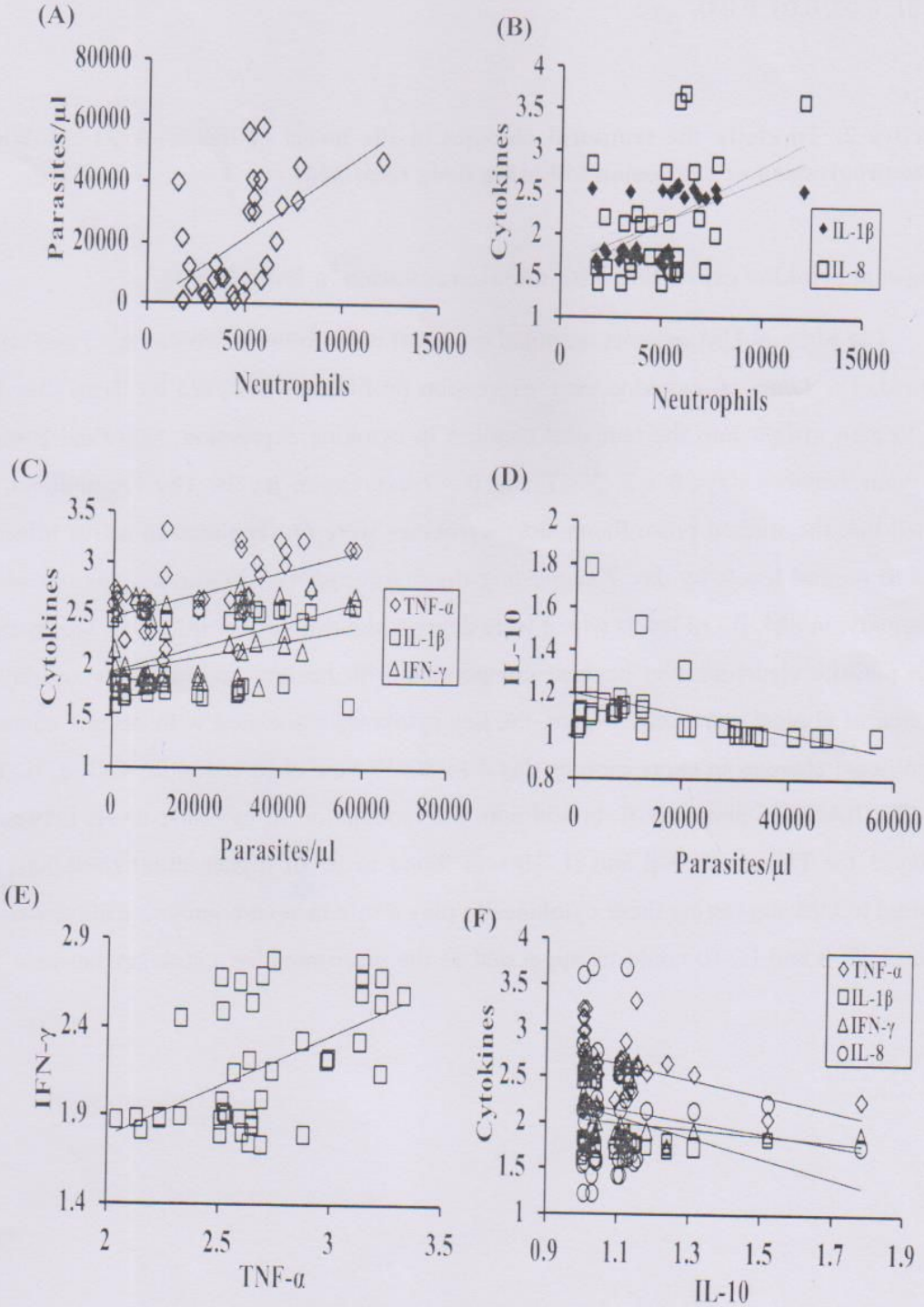


Figure 5: Correlation between neutrophils, parasitemia and expression of cytokines in SM (A-F). [(A) r=0.491, p=0.003 (B) r=0.412, 0.423, p=0.01, 0.01 (C) r=0.522, 0.394, 0.543, p=0.002,

0.02, 0.001 (D) r=-0.546, p=0.001 (E) r=0.359, p=0.03 (F) r=-0.408, -0.394, -0.368, -0.354, p=0.01, 0.02, 0.03, 0.04].

Objective 2: To study the temporal changes in the levels of the relevant cytokines with disease progression or regression following drug treatment

Changes in cytokine expression with disease resolution in SM and UM

The SM and UM patients recruited on day 0 were followed up on day 3 and day 7 post treatment. The temporal cytokine gene expression profile was analyzed by Real-Time PCR. In order to gain insight into the temporal changes in cytokine expression, statistical comparisons were made between days 0 - 3, 3 - 7 and 0 - 7 expression levels. The longitudinal analysis revealed that the studied pro-inflammatory cytokines were up regulated in active infection and tended to normal levels by day 7 suggesting the involvement of multiple cytokines in disease. Interestingly, in SM, IL-10 levels which were depressed during active infection, increased on day 3 with parasite clearance and became comparable with healthy controls only on day 7 with resolution of clinical symptoms. Again, the key cytokines associated with disease characterized by significant changes in expression by day 3 ($p<0.05$) were observed to be TNF-α, IL-1β, IFN-γ, IL-12α, RANTES and TGF-β. In addition, the modulation of cytokine levels between day 0 and day 3 for TNF-α, TGF-β and IL-10 was found to be of higher order ($p<0.0001$) in SM compared to UM suggesting these cytokines to play a role in severe malaria pathogenesis. Thus, TNF-α, TGF-β and IL-10 could be suggested as the discriminating cytokines between SM and UM.

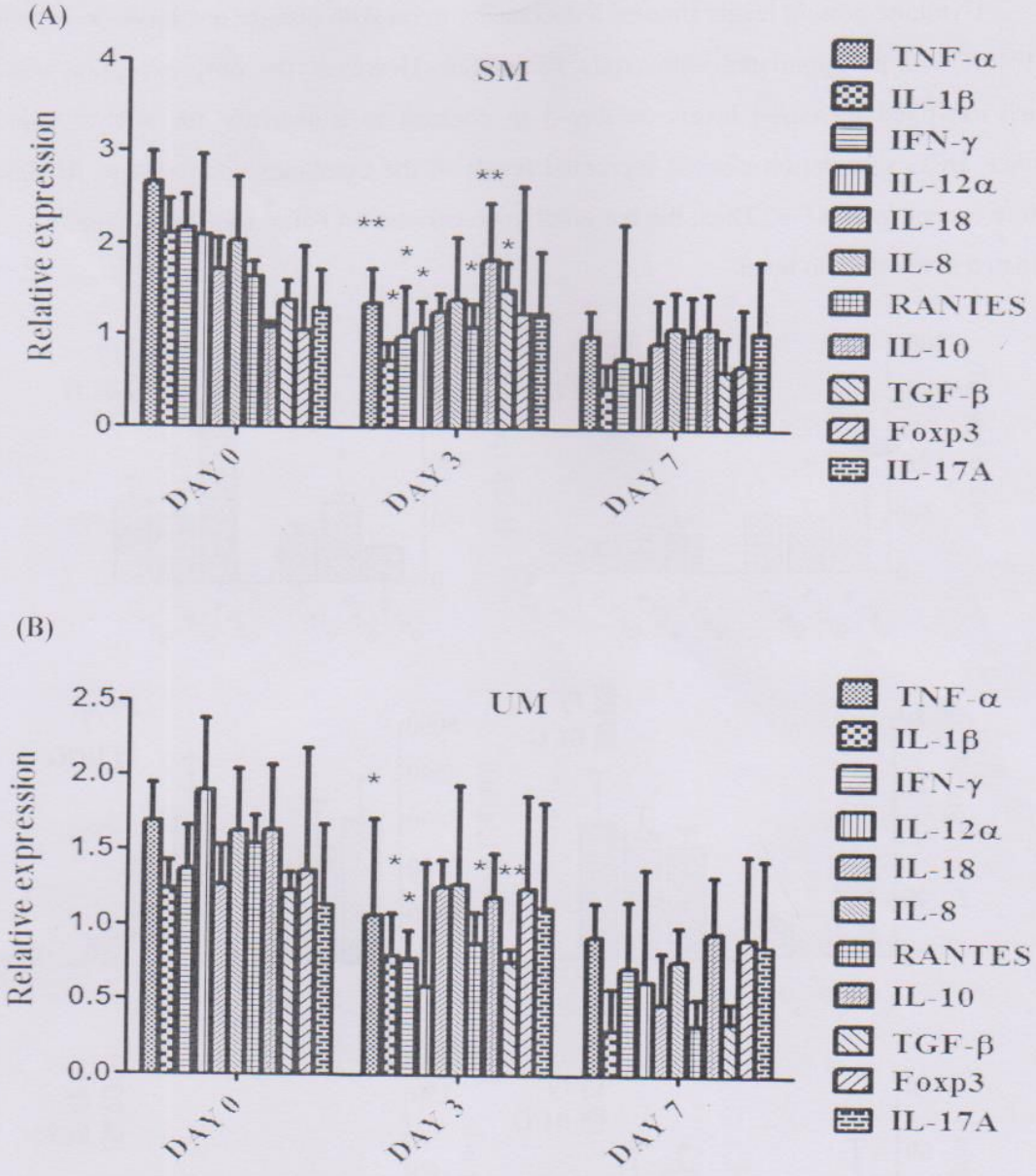


Fig 6: mRNA expression profile of cytokines/chemokines with disease resolution in SM (A) and UM (B). The mean relative expression was calculated with $2^{-\Delta\Delta Ct}$ formula using healthy control as the calibrator. Statistical comparisons were made between days 0 - 3, 3 - 7 and 0 - 7 expression levels by Paired t-test. Single and double asterisks indicate significant changes in day 3 expression of $p<0.05$ and $p<0.0001$ respectively.

Cytokine protein levels in UM

Cytokine protein levels showed a decreasing trend with disease resolution in supernatants of PBMC culture stimulated with crude *Pf* antigen. However, the only exception was IL-1β which exhibited increased levels on day 3 in contrast to a decrease for mRNA expression. Further, BCG stimulation elicited increased levels of the cytokines compared to *Pf* stimulated culture except for TNF-α. Thus, the temporal gene expression pattern of the studied cytokines is validated at the protein level.

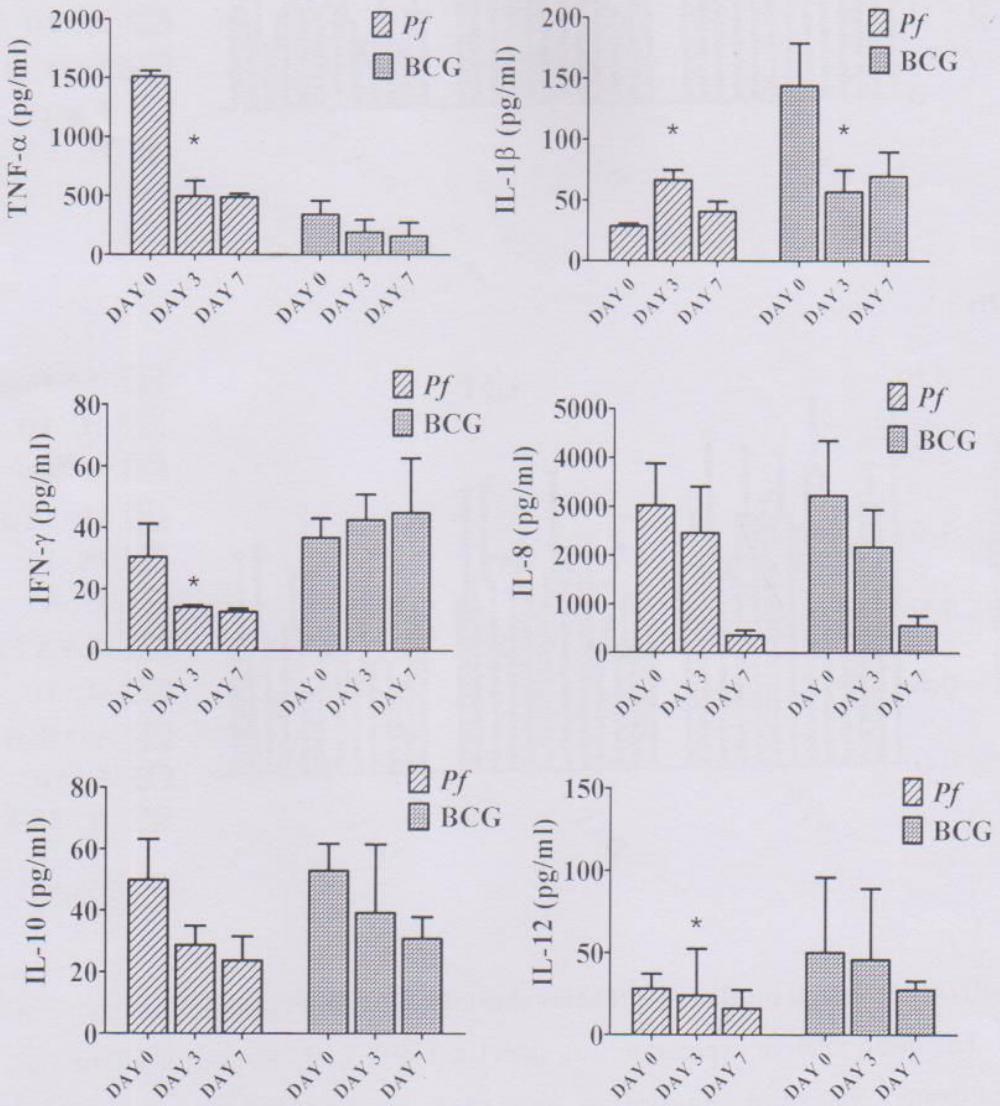


Figure 7: Cytokine/chemokine protein levels in supernatants of cell cultures stimulated with 40μg/ml of crude *Pf* antigen and BCG. Asterisks indicate significant changes in day 3 expression compared to day 0 ($p<0.05$). Error bars represent standard deviation of the mean.

Temporal mRNA expression profile of cytokines in stimulated PBMCs

Cytokine gene expression in *Pf* stimulated PBMCs was similar as that at the protein level except IL-1β which differed in day 3 data (Figure 18). Thus, the longitudinal changes in cytokine mRNA expression in *Pf* stimulated PBMCs was similar to blood mRNA levels.

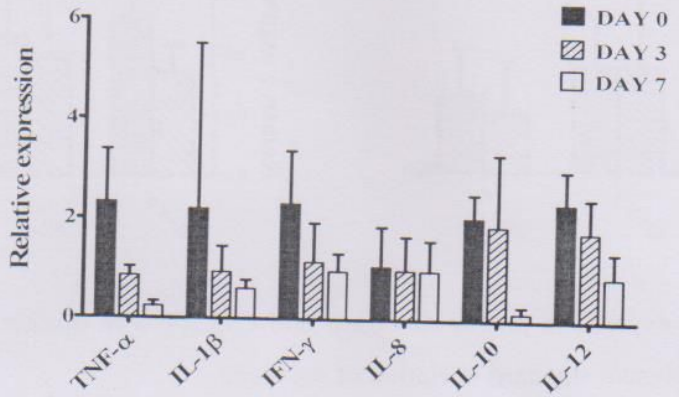


Figure 8: Cytokine/chemokine mRNA expression from PBMCs stimulated with crude *Pf* antigen. Error bars represent standard deviation of the mean.

Objective 3: To compare the expression of Foxp3 gene in complicated and uncomplicated malaria cases

Since Foxp3 is an important marker of T-reg cell development and function, so its expression was analyzed to assess the involvement of T-reg cells in malaria pathogenesis. Results revealed that SM group exhibited down regulation in the expression of Foxp3 on day 0 compared to UM, albeit not significant. Further, there was a modest increase on day 3 expression levels in SM which then attained normal levels by day 7 suggesting suppression of T-reg cell activation during malaria severity. In contrast, in UM, there was comparable expression between day 0 and day 3 followed by decrease to levels as that of healthy controls by day 7.

Further, there was increased expression of CTLA4, cell surface marker of T-reg cells in UM compared to SM on day 0. A slight increase in expression on day 3 was observed in SM similar to that demonstrated for Foxp3 providing further support to depressed T-reg cell activity in SM. Thus, low Foxp3 and CTLA4 expression in SM supported by undetectable levels of IL-2

suggests that T-reg cells are unlikely to be the cell source of increased TGF-β as IL-2 is known to be important in Foxp3 induction.

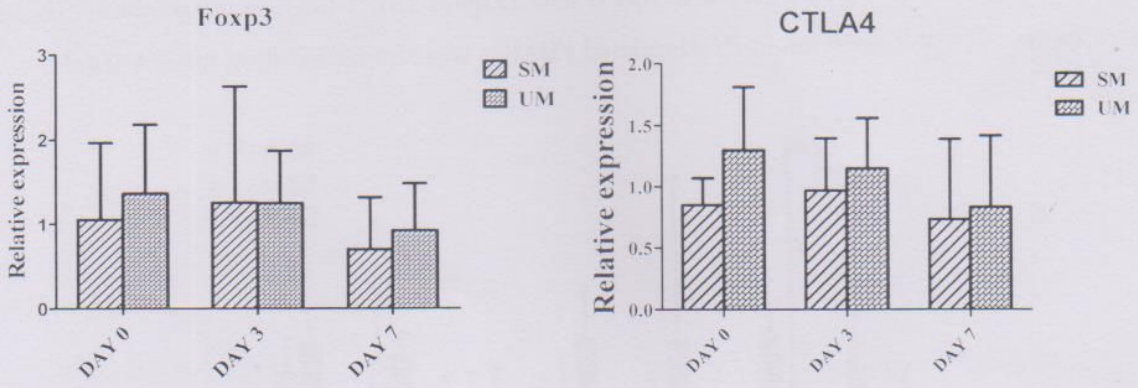


Figure 9: mRNA expression profile of Foxp3 and CTLA4 with disease resolution in SM and UM. Error bars represent standard deviation of the mean.

B2. Summary and Conclusions of the Progress made:

Longitudinal follow-up study revealed increased levels of pro-inflammatory cytokines counterbalanced by correspondingly increased anti-inflammatory cytokines in UM during active infection followed by decrease to normal levels with parasite clearance and resolution of clinical symptoms. In contrast to the balanced cytokine response in UM, an over exuberant pro-inflammatory cytokine response with decreased IL-10 levels was observed in SM. Further, a negative correlation of IL-10 levels with parasitemia and pro-inflammatory cytokines was obtained in SM. Even more interesting was an increase in IL-10 levels on day 3 in absence of parasitemia. This observation hints at the possible modulation of host cytokine response by parasite factors. At this point, we were prompted to speculate that down regulated levels of IL-10 and hence increased pro-inflammatory cytokines are exploited by falciparum for its own advantage since endothelial activation for the process of sequestration is known to be facilitated by pro-inflammatory cytokines. Another point worth mentioning is that TGF-β, expression of which was although comparatively higher in SM, failed to counter regulate an exaggerated pro-inflammatory cytokine response further supporting the already established indispensable role of IL-10 in prevention of immunopathology. Further, the very low levels of IL-2 along with lowly

expressed Foxp3 in SM indicate cells other than Foxp3+ T-regulatory cells to be involved in increased TGF-β secretion. In further support of their involvement in severe pathogenesis, neutrophils were found to be in strong positive correlation with parasitemia and levels of IL-1β, another of its activating factors. As one of the future directives, it would thus be imperative to study the neutrophil inflammatory pathway in order to have a detailed understanding of their role in malaria severity.

In conclusion, our data suggests failure of mechanisms that regulate the cytokine balance as indicated by decreased IL-10 gene expression which resulted in exaggerated inflammatory response in SM. Longitudinal changes in the cytokine levels also provided evidence for the role of TNF-α, IL-10 and TGF-β as the discriminating cytokines between SM and UM. Further, we hypothesize that immunosuppression of T helper arm and manipulation of innate cells and pathways by parasite factors appear to be the major factors contributing to heightened inflammation and disease severity in *Pf* malaria. In addition, a significant role of neutrophils in disease severity was observed which necessitates the importance to understand the host-pathogen interactions that would help design therapeutic interventions to provide optimal pathogen killing with minimal host damage.

B3. New Leads Obtained, if any:

The new leads obtained from the study are summarized below -

1. TNF-α, IL-10 and TGF-β discriminated between severe and uncomplicated malaria suggesting these cytokines to play a role in severe malaria pathogenesis.
2. An over exuberant pro-inflammatory cytokine response with decreased IL-10 levels in SM followed by an increase in levels with resolution of parasitemia hints at the possible modulation of host cytokine response by parasite factors.
3. The study also suggests role of neutrophils with increased IL-8 and IL-1β levels in severe inflammation in malaria.

B4. Details of Publications & Patents, if any:

1) Mahanta, A., Kar, S.K., Kakati, S. & Baruah, S. Heightened inflammation in severe malaria is associated with decreased IL-10 expression levels and neutrophils. *Innate Immun* 2014; e-pub ahead of print 2 December 2014; doi: 10.1177/1753425914561277.

2) Mahanta, A., Kakati, S. & Baruah, S. The association of IL-8-251T/A polymorphism with complicated malaria in Karbi Anglong district of Assam. *Cytokine* 2014; 65: 210-216.

Section-C: Details of Grant Utilization

C1. Equipment Acquired or Placed Order with Actual Cost:

1) ThermoFischer CO_2 Incubator was procured at Rs. 2,75,444
2) Eppendorf Refrigerated Centrifuge was procured at Rs. 3,77,850
3) APPLIED BIOSYSTEM Step One Plus Real Time PCR was procured at Rs. 13,18,111
4) Eppendorf Automated Liquid Handler was procured at Rs. 12,76,800

C2. Manpower Staffing and Expenditure Details:

One JRF recruited at Tezpur University
Expenditure: Rs. 5,39,896/- from 16.06.11 to 27.03.15
*Due – 47,609
One Project Assistant recruited at Tezpur University
Expenditure: Rs 90,933/- from 21.09.11 to 21.09.12
One Project Assistant recruited at Tezpur University
Expenditure: Rs 30,667/- from 06.06.13 to 01.11.13
One Field JRF recruited at Tezpur University
Expenditure: Rs. 22,452/- from 05.07.12 to 01.09.12
One Field Clinician recruited at Tezpur University
Expenditure: Rs. 30,000/- from 01.06.12 to 31.08.12

One SRF recruited at AMCH
Expenditure: Rs.2,48,160/- till 31st March 2014

C3. Details of Recurring Expenditure:

1. Expenditure under Manpower: Rs 7,13,948/-
2. Expenditure under Consumables: Rs. 1,009,626
3. Expenditure Under travel: Rs. 120,076
   *Due- Rs. 12,930
4. Expenditure under Contingency: Rs. 96,536
   *Due- Rs. 18,250
5. Expenditure under Overhead: Rs. 138,440

Total recurring expenditure: Rs. 5,326,831

S K Kar

Signature of Co-Principal Investigator
Investigator
Prof. S.K. Kar (KIIT, Bhubaneshwar)
Dibrugarh)

Signature of Co- Principal

Prof. Sanjeeb (AMCH,

Signature of Principal Investigator
Prof. S. Baruah, Tezpur University

FINAL CONSOLIDATED STATEMENT OF EXPENDITURE (FOR FINAL SETTLEMENT OF ACCOUNTS)

1. Title of the Project	: Longitudinal analysis of changes in cytokine profiles of malaria patients at different stages of treatment and disease resolution: Understanding the molecular basis of cure and malarial pathology
2. Sanctioned Project Cost	: 66.66 Lakhs (57.56 Lakhs for Tezpur University)
3. Revised cost, if any	: N/A
4. Duration of the project	: 3 years
5. Sanction Order No. & Date	: No. BT/CP/11/NE/TBP/2010 dated 28.03.11
6. Date of commencement of Project	: 28/03/2011
7. Extension, if any	: March 2014 - March 2015
8. Date of completion of project	: 27th March 2015

Details of grants, expenditure and balance

Sl. No	Heads	Sanctioned Cost	Year-wise Releases made (financial year-wise; in Rs.)					Year-wise Expenditure made (financial year-wise; in Rs.)					
			1st yr (01/04/11 - 31/03/12)	2nd yr (01/04/12 - 31/03/13)	3rd Yr (01/04/13 - 31/03/14)	4th Yr (01/04/14 - 31/03/15)	Total	1st yr (01/04/11 - 31/03/12)	2nd yr (01/04/12 - 31/03/13)	3rd Yr (01/04/13 - 31/03/14)	4th Yr (01/04/14 - 31/03/15)	Total	Balance
		A. Non-recurring											
1	Equipment	3,262,000	1,277,000	1,985,000			3,262,000	0	1,276,800	1,971,405		3,248,205	13,795
		B. Recurring											
1	Manpower	894,000	328,000	0	323,000	63,000	714,000	164,933	158,452	271,667	118,896	713,948	52
2	Consumabl	1,100,000	800,000	0	190,000	20,000	1,010,000	318,710	356,950	309,805	24,161	1,009,626	374
3	Travel	150,000	50,000	0	50,000	20,000	120,000	26,535	32,906	38,391	22,244	120,076	-76
4	Contingenc	150,000	50,000	0	45,000	0	95,000	17,842	27,185	51,509	0	96,536	-1536
5	Overhead	200,000	100,000	0	40,000	0	140,000	50,000	48,822	39,618	0	138,440	1560
	Total	5,756,000	2,605,000	19,85,000 + 20,26,980** = 40,11,980	6,48,000 + 21,10,865**= 27,58,865	1,03,000 + 76,470**= 1,79,470	5,341,000	578,020	1,901,115	2,682,395	165,301	5,326,831	14,169

(PROJECT INVESTIGATOR)

(FINANCE OFFICER)

(HEAD OF THE INSTITUTE)

Registrar
Tezpur University

Appendix-B

Utilisation Certificate

(for the financial year 1st April 2011-31st March 2012)

(Rs. in Lakhs)

1. Title of the Project/Scheme:
Longitudinal analysis of changes in cytokine profiles of malaria patients at different stages of treatment and disease resolution: Understanding the molecular basis of cure and malarial pathology/ DBT-Twining

2.	Name of the Organisation:	Tezpur University
3.	Principal Investigator:	Dr. Shashi Baruah
4.	Deptt. of Biotechnology sanction order No. & date of sanctioning the project:	No. BT/CP/11/NE/TBP/2010 dated 28.03.11
5.	Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given:	N/A
6.	Amount received from DBT during the financial year (*please give No. and dates of sanction orders showing the amounts paid)*:	Rs.26,05,000/- (No. BT/CP/11/NE/TBP/2010 dated 28.03.11)
7.	Other receipts/interest earned, if any, on the DBT grants:	N/A
8.	Total amount that was available for expenditure during the financial year:	Rs. 26,05,000/-
9.	Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed):	Rs. 5,78,020
10.	Unspent balance refunded, if any *(Please give details of cheque No. etc.):*	N/A
11.	Balance amount available at the end of the financial year:	Rs. 20,26,980/-

12. Amount allowed to be carried forward to the next financial year vide letter No. & date: N/A

1. Certified that the amount of Rs. 5,78,020 mentioned against col. 9 has been utilised on the project/scheme for the purpose for which it was sanctioned and that the balance of Rs. 20,26,980/- remaining unutilized at the end of the year will be adjusted towards the grants-in-aid payable during the next year.

2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

Kinds of checks exercised:

1.

2.

3.

4.

5.

02/02/15

(PROJECT INVESTIGATOR)

17.2.15

(FINANCE OFFICER)

Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)

(To be countersigned by the DBT Officer-in-charge)

Appendix-B

Utilisation Certificate

(for the financial year 1st April 2012-31st March 2013)

(Rs. in Lakhs)

1. Title of the Project/Scheme:
Longitudinal analysis of changes in cytokine profiles of malaria patients at different stages of treatment and disease resolution: Understanding the molecular basis of cure and malarial pathology/ DBT-Twining

2.	Name of the Organisation:	Tezpur University
3.	Principal Investigator:	Dr. Shashi Baruah
4.	Deptt. of Biotechnology sanction order No. & date of sanctioning the project:	No. BT/CP/11/NE/TBP/2010 dated 28.03.11
5.	Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given:	Rs. 20,26,980
6.	Amount received from DBT during the financial year (*please give No. and dates of sanction orders showing the amounts paid)*:	Rs. 19,85,000 (Corrigendum dated 13.04.2012)
7.	Other receipts/interest earned, if any, on the DBT grants:	N/A
8.	Total amount that was available for expenditure during the financial year:	Rs. 40,11,980
9.	Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed):	Rs. 19,01,115
10.	Unspent balance refunded, if any *(Please give details of cheque No. etc.):*	N/A
11.	Balance amount available at the end of the financial year:	Rs. 21,10,865

12. Amount allowed to be carried forward to the next financial year vide letter No. & date: N/A

1. Certified that the amount of Rs. 19,01,115 mentioned against col. 9 has been utilised on the project/scheme for the purpose for which it was sanctioned and that the balance of Rs. 21,10,865 remaining unutilized at the end of the year will be adjusted towards the grants-in-aid payable during the next year.

2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

Kinds of checks exercised:

1.

2.

3.

4.

5.

17-7-15

(PROJECT INVESTIGATOR)

17-7-15

(FINANCE OFFICER)

Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)

Registrar
Tezpur University

(To be countersigned by the DBT Officer-in-charge)

Appendix-B

Utilisation Certificate

(for the financial year 1st April 2013-31st March 2014)

(Rs. in Lakhs)

1. Title of the Project/Scheme:
Longitudinal analysis of changes in cytokine profiles of malaria patients at different stages of treatment and disease resolution: Understanding the molecular basis of cure and malarial pathology/ DBT-Twining

2.	Name of the Organisation:	Tezpur University
3.	Principal Investigator:	Dr. Shashi Baruah
4.	Deptt. of Biotechnology sanction order No. & date of sanctioning the project:	No. BT/CP/11/NE/TBP/2010 dated 28.03.11
5.	Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given:	Rs. 21,10,865
6.	Amount received from DBT during the financial year (*please give No. and dates of sanction orders showing the amounts paid)*:	Rs. 6,48,000 (Corrigendum dated 02.05.2013)
7.	Other receipts/interest earned, if any, on the DBT grants:	N/A
8.	Total amount that was available for expenditure during the financial year:	Rs. 27,58,865
9.	Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed):	Rs. 26,82,395
10.	Unspent balance refunded, if any *(Please give details of cheque No. etc.):*	N/A
11.	Balance amount available at the end of the financial year:	Rs. 76,470

12. Amount allowed to be carried forward to the next financial year vide letter No. & date: N/A

1. Certified that the amount of Rs. 26,82,395 mentioned against col. 9 has been utilised on the project/scheme for the purpose for which it was sanctioned and that the balance of Rs. 76,470 remaining unutilized at the end of the year will be adjusted towards the grants-in-aid payable during the next year.

2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

Kinds of checks exercised:

1.

2.

3.

4.

5.

(PROJECT INVESTIGATOR)

12.7.15

(FINANCE OFFICER)

Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)

Tezpur University

(To be countersigned by the DBT Officer-in-charge)

Appendix-B

Utilisation Certificate

(for the financial year 1st April 2014-27th March 2015)

(Rs. in Lakhs)

1. Title of the Project/Scheme:
Longitudinal analysis of changes in cytokine profiles of malaria patients at different stages of treatment and disease resolution: Understanding the molecular basis of cure and malarial pathology/ DBT-Twining

2.	Name of the Organisation:	Tezpur University
3.	Principal Investigator:	Dr. Shashi Baruah
4.	Deptt. of Biotechnology sanction order No. & date of sanctioning the project:	No. BT/CP/11/NE/TBP/2010 dated 28.03.11
5.	Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given:	Rs. 76,470
6.	Amount received from DBT during the financial year (*please give No. and dates of sanction orders showing the amounts paid)*:	Rs. 1,03,000 (Corrigendum dated 08.09.2014)
7.	Other receipts/interest earned, if any, on the DBT grants:	N/A
8.	Total amount that was available for expenditure during the financial year:	Rs. 1,79,470
9.	Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed):	Rs. 1,65,301
10.	Unspent balance refunded, if any *(Please give details of cheque No. etc.):*	N/A
11.	Balance amount available at the end of the financial year:	Rs. 14,169

12. Amount allowed to be carried forward to the next financial year vide letter No. & date: N/A

1. Certified that the amount of Rs. 1,65,301 mentioned against col. 9 has been utilised on the project/scheme for the purpose for which it was sanctioned and that the balance of Rs. 14,169 remaining unutilized at the end of the year will be adjusted towards the grants-in-aid payable during the next year.

2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

Kinds of checks exercised:

1.

2.

3.

4.

5.

22/07/15

(PROJECT INVESTIGATOR)

17.7.15

(FINANCE OFFICER)

Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)

Registrar
Tezpur University

(To be countersigned by the DBT Officer-in-charge)

Annexure A

Due- Drawn Statement

Name of the Project Staff	Month and Year	Due	Drawn	Difference
1) Ms. Anusree Mahanta (SRF)	15th December 2014 -27th March 2015	Rs. 47,609	Nil	

Project Head	Year	Due
Contingency	2014	Rs. 18,250
Travel	2014	Rs. 12,930

02/07

(Signature of Principal Investigator)

7.7.15

(Signature of Accounts Officer)

Finance Officer
Tezpur University

(SIGNATURE OF HEAD OF THE INSTITUTE)

Registrar
Tezpur University

Appendix-A

Details of Assets acquired wholly or substantially out of Govt. grants Register to be maintained by Grantee Institution

Name of the Sanctioning Authority: DEPARTMENT OF BIOTECHNOLOGY (NER DIVISION)

1. Sl. No. 388
2. Name of the Grantee Institution TEZPUR UNIVERSITY
3. No. & Date of sanction order No.BT/CP/11/NE/TBP/2010 dated 28.03.2011
4. Amount of the sanctioned grant 57,56,000/-
5. Brief purpose of the grant To investigate the cytokine profiles in malaria with progression and resolution of disease and parasitemia which is required to reveal the complex network of immune responses and risk of disease. The outcome of this study may be exploited in routine detection of some cytokines that may be relevant to diagnosis and prognosis of the various clinical conditions involved in falciparum malaria.
6. Whether any condition regarding the right of ownership of Govt. in the Property or other assets acquired out of the grant was incorporated in the grant-in-aid sanction order. Yes
7. Particulars of assets actually credited or acquired.

Item	Expenditure	Remarks
ThermoFischer CO2 INCUBATOR AND ACCESSORIES	2,75,444	Payment made
EPPENDORF REFRIGERATED CENTRIFUGE	3,77,850	Payment made
APPLIED BIOSYSTEM STEP ONE PLUS REAL TIME PCR	13,18,111	Payment made
EPPENDORF'S AUTOMATED LIQUID HANDLER (MODEL EPMOTION 5070)	12,76,800	Payment made

8. Value of the assets as on	a) Rs. 2,75,444 for ThermoFischer CO_2 INCUBATOR on 06.11.13 b) Rs. 3,77,850 for EPPENDORF'S REFRIGERATED CENTRIFUGE ON 08.08.13 c) Rs. 13,18,111 for APPLIED BIOSYSTEM STEP ONE PLUS REAL TIME PCR on 21.09.12 d) Rs. 12,76,800 for EPPENDORF'S AUTOMATED LIQUID HANDLER (MODEL EPMOTION 5070) on 05.01.12
9. Purpose for which utilised at present	The Real Time PCR machine is being used for cytokine gene expression studies. The centrifuge is being used for settling down real time PCR reactions. The incubator is being used for cell culture work. The liquid handler is being used for accurate and precise pipetting of large number of samples.
10. Encumbered or not	No
11. Reasons, if encumbered	Not applicable
12. Disposed of or not	Not applicable
13. Reasons and authority, if any, for Disposal	Not applicable
14. Amount realised on disposal	Not applicable
15. Remarks	_____

12/07

(PROJECT INVESTIGATOR)

17.7.15

(FINANCE OFFICER)

FINANCE OFFICER
Tezpur University

(HEAD OF THE INSTITUTE)

Registrar
Tezpur University

* List of equipment purchased indicating the item wise costs may please be provided.

Manpower Staffing Details (1st April 2011 – 31st March 2012)

NAME OF THE PERSON	NAME OF THE POST	DATE OF JOINING	DATE OF LEAVING	TOTAL MONTHLY SALARY	TOTAL SALARY PAID DURING THE FINANCIAL YEAR	TOTAL SALARY PAID DURING PROJECT PERIOD
Anusree Mahanta	JRF	16.06.11		12000	1,14,000	1,14,000
Biswajit Saikia	Project Assistant	21.09.11		8000	50,933	50,933

02/07

(Signature of Principal Investigator)

12.7.12

(Signature of Accounts Officer)

Finance Officer
Tezpur University

(SIGNATURE OF HEAD OF THE INSTITUTE)

Registrar
Tezpur University

Manpower Expenditure Details (28th March 2011 – 31st March 2012):

SANCTIONED POSTS	NUMBER	SCALE OF PAY	ANNUAL OUTLAY	OUTLAY FOR THE ENTIRE PERIOD	REVISED SCALE, IF ANY	REVISED ANNUAL OUTLAY	REVISED PROJECT OUTLAY	ACTUAL RELASES BY DBT	ACTUAL EXPENDITURE	BALANCE
JRF	1	Rs.12000 per Month	144000	6,60,000				2,11,000	1,14,000	97,000
Project Assistant	1	Rs.8000 Per Month	96000	2,34,000				1,17,000	50,933	66,067

(Signature of Principal Investigator)

(Signature of Accounts Officer)

Finance Officer
Tezpur University

(SIGNATURE OF HEAD OF THE INSTITUTE)

Registrar
Tezpur University

Manpower Staffing Details (1st April 2012 – 31st March 2013)

NAME OF THE PERSON	NAME OF THE POST	DATE OF JOINING	DATE OF LEAVING	TOTAL MONTHLY SALARY	TOTAL SALARY PAID DURING THE FINANCIAL YEAR	TOTAL SALARY PAID DURING PROJECT PERIOD
Anusree Mahanta	JRF	16.06.11		12000	96,000	2,10,000
Biswajit Saikia	Project Assistant	21.09.11	21.09.12	8000	40,000	90,933
Rowell Rongphar	Field JRF	05.07.12	01.09.12	12000	22452	22,452

(Signature of Principal Investigator)

12.7.13

(Signature of Accounts Officer)

Finance Officer
Tezpur University

(SIGNATURE OF HEAD OF THE INSTITUTE)

Registrar
Tezpur University

Manpower Expenditure Details (1st April 2012 – 31st March 2013):

SANCTIONED POSTS	NUMBER	SCALE OF PAY	ANNUAL OUTLAY	OUTLAY FOR THE ENTIRE PERIOD	REVISED SCALE, IF ANY	REVISED ANNUAL OUTLAY	REVISED PROJECT OUTLAY	ACTUAL RELASES BY DBT	ACTUAL EXPENDITURE	BALANCE
JRF	1	Rs.12000 per Month	144000	6,60,000				97,000	1,18,452	-21,452
Project Assistant	1	Rs.8000 Per Month	96000	2,34,000				66,067	40,000	26,067

(Signature of Principal Investigator)

12.7.15

(Signature of Accounts Officer)

Finance Officer
Tezpur University

(SIGNATURE OF HEAD OF THE INSTITUTE)

Registrar
Tezpur University

Manpower Staffing Details (1st April 2013 – 31st March 2014)

NAME OF THE PERSON	NAME OF THE POST	DATE OF JOINING	DATE OF LEAVING	TOTAL MONTHLY SALARY	TOTAL SALARY PAID DURING THE FINANCIAL YEAR	TOTAL SALARY PAID DURING PROJECT PERIOD
Anusree Mahanta	SRF	16.06.11		Rs.12000 till May 2013 and Rs.14,000 from June 2013	2,11,000	4,21,000
Dr. Irene Beypi	Field * clinician	01.06.12	31.08.12	10000	30,000	30,000
Sansuma Basumatary	Project Assistant	06.06.13	01.11.13	8000	30,667	30,667

(Signature of Principal Investigator)

17.7.15

(Signature of Accounts Officer)

Finance Officer
Tezpur University

(SIGNATURE OF HEAD OF THE INSTITUTE)

Registrar
Tezpur University

* PART-TIME APPOINTMENT

Manpower Expenditure Details (1st April 2013 – 31st March 2014):

SANCTIONED POSTS	NUMBER	SCALE OF PAY	ANNUAL OUTLAY	OUTLAY FOR THE ENTIRE PERIOD	REVISED SCALE, IF ANY	REVISED ANNUAL OUTLAY ANNUAL	REVISED PROJECT OUTLAY	ACTUAL RELASES BY DBT	ACTUAL EXPENDITURE	BALANCE
SRF	1	Rs.12000 per Month	144000	6,60,000	14,000 (Promotion to SRF)	1,68,000		1,76,000	2,11,000	-35,000
Project Assistant	1	Rs.8000 Per Month	96000	2,34,000				1,17,000	30,667	86,333
Field clinician	1	Rs.10000 per month						30,000	30,000	-

(Signature of Principal Investigator)

17.2.15

(Signature of Accounts Officer)

Finance Officer
Tezpur University

(SIGNATURE OF HEAD OF THE INSTITUTE)

Registrar
Tezpur University

Manpower Staffing Details (1st April 2014 – 27th March 2015)

NAME OF THE PERSON	NAME OF THE POST	DATE OF JOINING	DATE OF LEAVING	TOTAL MONTHLY SALARY	TOTAL SALARY PAID DURING THE FINANCIAL YEAR	TOTAL SALARY PAID DURING PROJECT PERIOD
Anusree Mahanta	SRF	16.06.11	27.03.15	14000	1,18,896	5,39,896

(Signature of Principal Investigator)

17.7.15

(Signature of Accounts Officer)

Finance Officer
Tezpur University

(SIGNATURE OF HEAD OF THE INSTITUTE)

Registrar
Tezpur University

Manpower Expenditure Details (1st April 2014 – 27th March 2015):

SANCTIONED POSTS	NUMBER	SCALE OF PAY	ANNUAL OUTLAY	OUTLAY FOR THE ENTIRE PERIOD	REVISED SCALE, IF ANY	REVISED ANNUAL OUTLAY	REVISED PROJECT OUTLAY	ACTUAL RELASES BY DBT	ACTUAL EXPENDITURE	BALANCE
SRF	1	Rs.14000 per Month	168000					1,18,948	1,18.896	52

(Signature of Principal Investigator)

17.7.15

(Signature of Accounts Officer)

Finance Officer
Tezpur University

(SIGNATURE OF HEAD OF THE INSTITUTE)

Registrar
Tezpur University