Serial measurements of serum C-Reactive Protein (CRP) oscillate in cancer patients. Can CRP be used as a surrogate biomarker for immune regulation?

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Background: There have been many attempts to stimulate the cancer patient's immune system for therapeutic benefit – results have been variable and usually disappointing. Recent evidence suggests that anti-tumor immune responses are being continuously attenuated with regulatory T-lymphocytes (Tregs) playing a major role. Currently Treg manipulation is being explored on a number of fronts and knowing how to accurately target Tregs will be important in therapeutic intervention.

The immune response is known to be highly orchestrated, sequential and time dependent. Consequently, in trying to understand this seemingly complex biological process, close serial measurements are needed to accurately resolve the immune dynamics and kinetics.

The inflammatory marker serum CRP is known to rise and fall with initiation and termination of inflammatory responses. In addition, CRP can be elevated in the cancer patient and can rise with disease progression.

Preliminary assessments of this inflammatory marker have shown that cyclical variation in serum concentrations can often be detected in cancer patients and patients with other chronic inflammatory conditions such as HIV/AIDS (Figs 1 & 2).

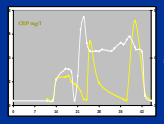


Fig 1. HIV Viral load / CRP fluctuations post cessation of HAART over 5 weeks indicates a cyclical CRP oscillation.

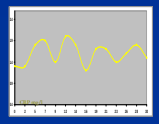


Fig 2. CRP fluctuations in an ovarian cancer patient over a 4 week period indicates a CRP periodicity of ~7 days.

Hypothesis: We hypothesize that Treg activity varies in a cyclical fashion and that this activity may counter-oscillate with changes in CRP. We further hypothesize that variation in immune regulatory activity may reflect periodicity in Treg proliferation.

Aim: In this study we sought to serially map over (up to) a 4 week period the fluctuations in acute phase reactants, certain cytokines and lymphocyte subsets in the late stage asymptomatic cancer patient. And further, to mathematically analyse the collected data to test for periodicity.

Methods: Serial measurements over a 4 week period were taken in ten (10) late-stage patients with various cancers. Assays included serum high sensitive CRP, SAA, IL10, TGF-b1 and two circulating T-lymphocyte subsets: CD4+/TGF-b1 secreting cells and CD4+CD25+/ Foxp3/CD127- cells. Approximately 20ml of blood was taken from each Patient every Monday, Wednesday and Friday over 4 weeks.

Mathematical Analysis Methods: The collected data were modeled mathematically using Monte-Carlo simulations applied to cumulative periodograms, and non-linear regression applied to a harmonic model to test for CRP periodicity and counter periodicity in immune regulatory cytokine TGF-b1. The Monte-Carlo analysis uses the serial data sets to investigate whether or not there is a periodic oscillation in serum CRP and TGF-beta. In each case, the null hypothesis is; there is no periodic oscillation – this is the statistical equivalent of the modeler having no prior expectation of what the result might be.

"If this (CRP) variation reflects Treg proliferation then carefully timed chemotherapy might be used to enhance anti-cancer immune responses by deleting these cells in a cycle-specific manner".

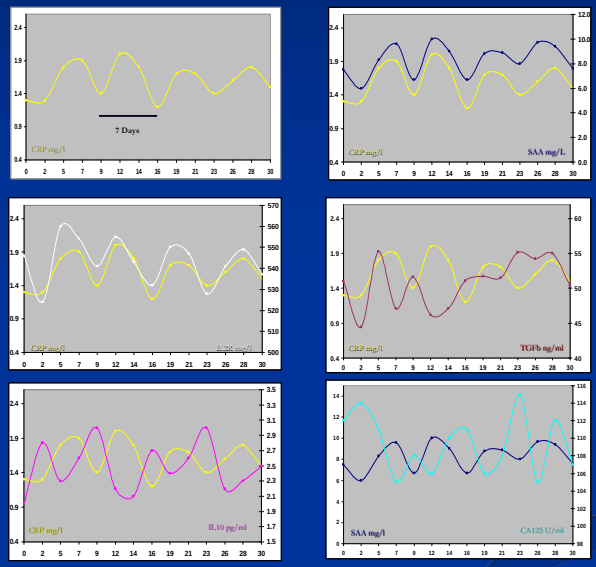


Fig 3. Patient FO. Serial acute phase marker, cytokine & cancer marker fluctuations in a late stage asymptomatic ovarian cancer patient (as marked) over a 4 week period indicating a periodicity of ~7 days consistent with the 6.41 days determined via the modeling analysis. See also Table 1.

Patient n=10	*Age*	*Sex*	*Cancer*	*Mean CRP mg/L*	*CRP range mg/L*	*Estimated CRP period (days)*	*Std Error*	*Estimated TGFb period (days)*	*Std Error*	*Estimated CRP/TGFb Relative cycle phase shift*
AP (see Fig 5b.)	47	M	Melanoma	36.24	29.17- 81.9	5.61	0.5844	6.56	0.299	0.223
CC	57	M	Melanoma	13.89	7.30 - 36.03	9.19	0.6670	ND	ND	ND
DM (see Fig 5c.)	78	M	Melanoma	18.55	4.10 - 76.8	7.33	0.4269	4.47	0.159	0.344
FO (see Figs 3.)	72	F	Ovarian	1.60	1.3 - 2.0	7.76	0.1855	7.76	1.038	0.728
GD (see Fig 5a.)	60	M	Colorectal	21.89	16.7 - 27.7	6.37	0.2863	7.63	0.580	0.221
KD	78	M	Melanoma	8.43	4.67 – 11.08	6.52	0.2664	7.97	0.587	0.655
LS	58	F	NSCLC	18.89	4.9 – 37.6	8.15	1.3808	ND	ND	ND
MB	75	F	Ovarian	73.20	5.6 – 97.5	3.14	0.1017	ND	ND	ND
MP	68	F	Melanoma	8.51	3.61 – 16.69	6.48	0.4744	6.92	0.546	0.644
RI	30	F	Melanoma	1.22	0.94 – 1.98	3.55	0.0522	6.04	0.397	0.425
Average						6.41 days $\alpha = 0.05$	0.57 (Qm)	7.76 days $\alpha = 0.05$	0.55 (Qm)	0.460 days (SE=).08 0.5 indicates half period phase shift (counter-oscillation)

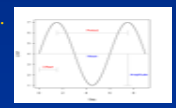
Table 1. Patient details, CRP and TGF-beta waveform analysis results. Qm = Quadratic mean

Mathematical Analysis Results: The Monte-Carlo analysis provides an indication that the patients serial data exhibits greater periodicity than would be reasonably expected to occur if the data were non periodic or random. From this stringent test, the fact that most of the data sets do is definitely noteworthy. Consequently the null hypothesis of no periodic oscillation should be rejected. In each case, having determined that there is statistically significant evidence against the null hypothesis of no periodic oscillation, the model can be used to investigate estimates of the period from wave form variables, as in Fig 4a & b. This approach gives opportunity to compare the CRP and TGF-beta interactions. The tabulated results of this analysis are presented in table 1. The alpha values indicate the probability of periodicity in a given data set. A low alpha value gives an indication of the confidence / probability of the existence of periodicity in the serial data set

a.

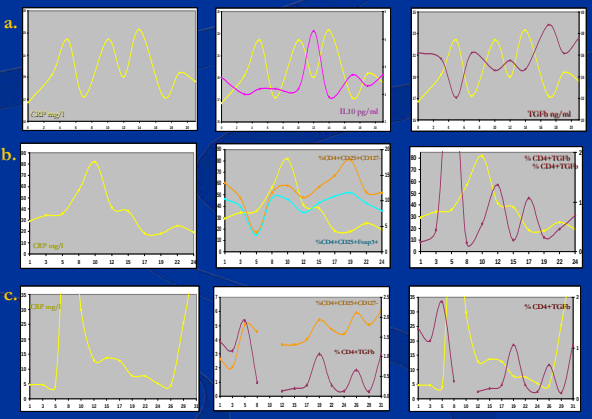
$$CRP_i = amplitude \times \sin\left(2\pi \times \left(\frac{day_i - offset}{period} + 0.25\right)\right) + mean + \epsilon_i$$

b.



Figs 4,(a) Wave form period equation and, (b) waveform variables

Findings: Seven out of ten patients returned statistically significant periodicity for CRP ($\alpha = 0.05$) according to simulation tests of the cumulative periodograms of their CRP traces. The average estimated period from the harmonic models was 6.41 days, and ranged from 3.14 to 9.19 days. Six of seven patients returned statistically significant periodicity for TGF-b1 ($\alpha = 0.05$), and the average estimated period from the harmonic models was 6.76 days, ranging from 4.47 to 7.97. For testing counter-oscillations the average ratio of the offset differences to the period for the seven patients comparing CRP to TGFb1 was 0.46 (SE = 0.08). This indicates that the CRP and TGF beta (serum or cellular) have similar but out of phase periodicities of approximately half a period.



Figs 5. a, b &c Patients GD, AP & DM. CRP, IL10, TGFb, CD4+CD25+/foxp3/CD127-, CD4+/TGFb, as marked

Conclusion: Serial measurements of CRP provide evidence for cyclical regulation of inflammation in cancer patients. Preliminary data suggests this may be associated with counter cyclical variation in the regulatory cytokine TGFb1. If this variation reflects Treg proliferation then carefully timed chemotherapy might be used to enhance anti-cancer immune responses by deleting these cells in a cycle-specific manner.

It is also possible that the random application of chemotherapy currently may be accidentally and fortuitously ablating Tregs for the benefit of the patient should the application occur at the correct point in this hypothesised regulated cycle. This may account for the low but consistent complete response rate seen in the late stage cancer patient.

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