# Improving Vaccine and Chemotherapies for Advanced Melanoma and Ovarian Cancer.

## Understanding Immune System Kinetics to Better Time Treatment.

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## Abstract

Recent evidence suggests that for a number of tumour types, an underlying persistent anti-tumour immune response is being continuously attenuated by the immune system's homeostatic regulatory mechanisms.

Immune suppression in the tumour micro-environment is emerging as a dominant therapeutic barrier. Although therapies for advanced malignancy have been disappointing, therapeutic vaccines appear promising agents.

C-Reactive Protein (CRP), an acute phase reactant/ opsonin is known to rise/ fall over several days with initiation/ termination of the immune response.

Using serial CRP measurements in late-stage cancer patients, we have recently been able to expose sequential and time dependent oscillations in the inflammatory/ immune response that may represent a homeostatic, repeating or cyclical process of tumour immune responsiveness then tolerance. The periodicity of these cycles appears to be reproducible at approximately 6-7 days.

By serially measuring CRP around the time of vaccination or chemotherapy, the position on the underlying immune curve can be established. Timing with respect to this cycle appears to be critical to modulating the immune system with each intervention, and pivotal to the clinical efficacy of therapy (1).

Using these methods, we have been able to correlate the timing of vaccination/ chemotherapy with the induction of clinical responses. Induction of complete responses, stable disease, slowed growth even with persistent metastatic disease, appears possible using these principles, thereby improving overall survival.

Timed chemotherapy using similar methods has recently been reported to be associated with improved patient clinical outcome by our collaborators (2). Furthermore, timed low dose cyclophosphamide is currently under investigation in ovarian cancer with promising initial results.

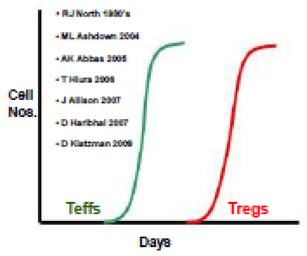
More accurately timed delivery of vaccine/ chemotherapy approaches might realistically induce therapeutic reversal of immune suppression in patients with metastatic melanoma, ovarian cancer and cancer generally. Patient data is presented here.

## Homeostatic regulated immune kinetics in cancer

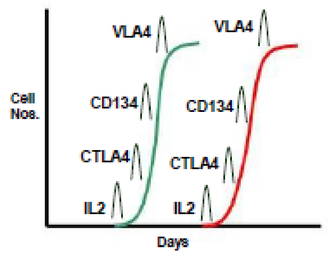

"Nature exists in a delicate balance, the immune system being no exception."

The immune response once triggered, is known to be a time dependent, sequential, orchestrated & homeostatic physiological process. This process is mediated via the timely interaction of cytokines, receptors and cell types. Understanding a dynamic process requires close serial measurements. Published evidence suggests the timing of therapy (various modalities) dictates therapeutic outcome in releasing the tumour immune response from homeostatic regulation in cancer.

### Similar clonal kinetics of Teffs & Tregs.



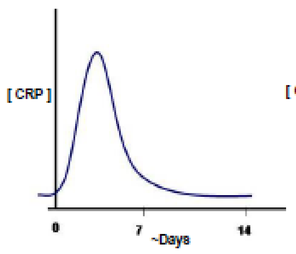

Reported sequential time dependent interaction of Teffs & Tregs following stimulation in a number of models.



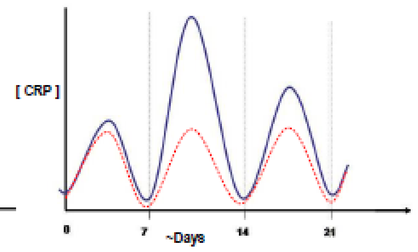

Reported bimodal transient expression / interaction of the same cytokines / receptors on both Teffs & Tregs.

## C- Reactive Protein (CRP) – a potential surrogate biomarker of immune kinetics

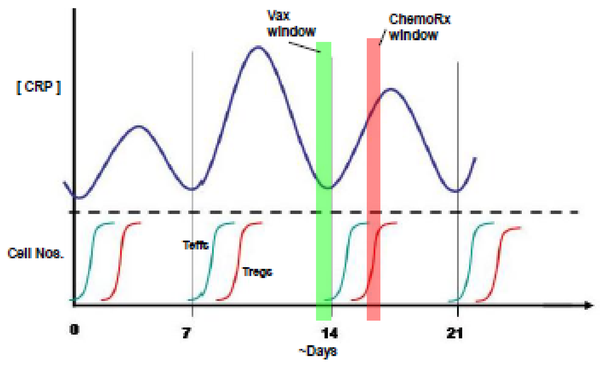
CRP, an opsonin and a non-specific functional analogue of Immunoglobulin mediates the adaptive immune response through the FC-gamma Receptors on DC's. This protein is known to rise/ fall over several days in the acute state with initiation/ termination of the immune response. We have observed that CRP is elevated and oscillating over an approximate 7 day repeating cycle in the late-stage cancer patient in all cancer types monitored so far.




CRP kinetics in the acute response




CRP kinetics in the late stage cancer patient - what we & collaborators have observed in various cancers. Amplitudes in the CRP oscillations may vary whilst periodicity remains almost fixed.




Hypothesized timed therapeutic interaction window of either Vaccine (green) or chemotherapy (red) with respect to CRP cycle to enhance tumour immune responsiveness via immune stimulation or Treg ablation

## Vaccinia Melanoma Cell Lysate (VMCL) vaccine therapy for advanced melanoma

VMCL Trial Summary

In 2000, we commenced treating advanced melanoma patients using a vaccine. Patients with stage IV melanoma have a predicted survival of 3-6 months, with a 1% chance of surviving for 4 years, by any current treatment. Our first vaccine treated patient is alive and disease-free almost 9 years later, and we now have 13 of 54 (24%) treated patients alive and well. 12 patients (22.2%) have survived for more than 18 months, and 5 of these are currently alive from 4 to 9 years - and this survival time will gradually increase as their time on the trial increases.

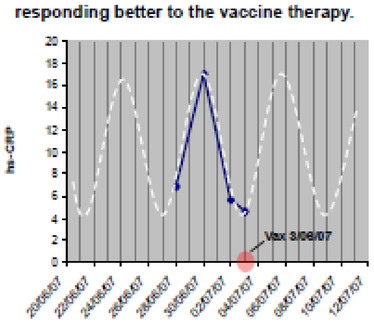
Data analysis to the end 2008 - random vaccination (N=54)

| Response Rates with VMCL Vaccine | |
|---|---|
| CR overall | 10 patients (18.5%) durable 2-8yrs |
| SD overall | 26 patients (48.2%) |
| PR | 8 patients (14.8%) |
| PD | 10 patients (18.5%) |

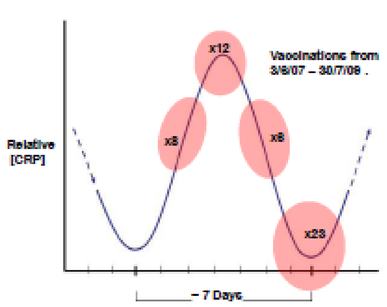
VCML benefits
- Low toxicity
- Better efficacy

### Timing Vaccine administration WRT CRP Cycle

Over the last 6 years, we have noticed a 'cyclical type' of pattern of disease growth in some vaccine treated patients. We also noticed that reducing the dose frequency appeared to reduce the effectiveness of the vaccine in controlling the melanoma growth rate; while increasing the frequency of doses could regain control. Over the last 3-4 years, we have been identifying background immune cycles using serial C-Reactive Protein (CRP) measurements in these patients in an effort to understand why responses in patients might vary. These studies have revealed that the patients that randomly receive their vaccine doses close to the trough of their CRP immune cycles, appear to be responding better to the vaccine therapy.




An example of serial monitoring hs-CRP the week before and the day of vaccination. Note how the cycle kinetics can be approximately resolved with a week of serial data.

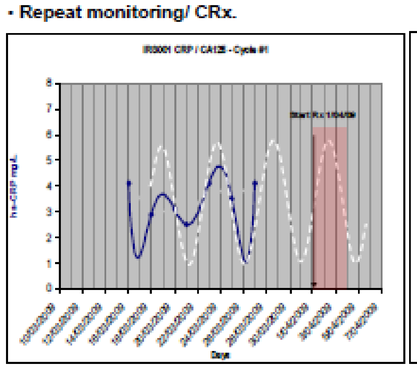



Number of vaccinations and their approximately position on the CRP cycle, either near a peak or a trough, etc. Patient #JM;Total vaccinations = 48.

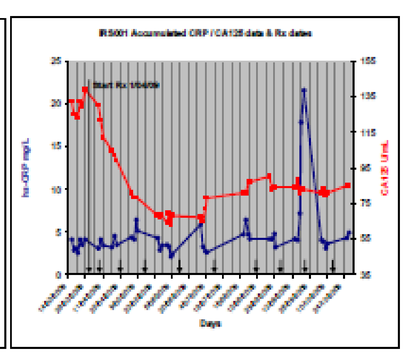
## Synchronising low dose oral Cyclophosphamide with the Immune Cycle in Ovarian Cancer

### Protocol Outline

- OvCa Patients who have previously failed two lines of chemotherapy / rising CA125
- Monitor hs-CRP serially for two weeks to detect cycle.
- Estimate cycle periodicity.
- Project forward to putative date/ time to initiate therapy, (therapeutic "sweet spot").
- Monitor response to therapy (CA125)
- Repeat monitoring/ CRx.




Mapping the pre-existing immune response and timing therapy




Treatment times & response to therapy (CA125)

### Rationale

The serial hs-CRP cycle is used as a surrogate marker of immune regulation kinetics to preferentially ablate synchronously dividing Tregs - disrupting tumour immune homeostasis to favour immune responsiveness/ tumour destruction (as described in mouse experiments).

### Dosing & Approximate Cost per treatment episode

Serial hs-CRP assay/ phlebotomy = ~ $400

2 x 50mg cyclophosphamide tablets on each of 3 consecutive days = < $3.00

## Conclusion

- Serial daily CRP measurements can identify time dependent cyclical immune kinetics in cancer patients.
- Single measurements alone would fail to resolve such kinetics and could be misleading.
- Vaccine/ chemotherapy timing requires serial CRP/ immune marker time-point analysis.
- Timing therapy with the immune response cycle appears to be important for improved treatment and clinical outcome.

## Implications

- Immune modulation appears possible using timed vaccine and/ or chemotherapy.
- Historically, the low rate of therapeutic successes (clinical responses and survival) could be due to random and fortuitous immune-modulation with chemotherapy.
- Therapeutic failures in both vaccines and chemotherapy might be due to induction of tolerance.
- Chemo-resistance may be an "artifact" of the inappropriate timing of therapy.
- Accurately timing therapy may improve responses with less agent and lower toxicity.
- A timely, "immune-targeted" approach might reduce costs and simplify cancer treatment.
- Standard approaches to treating advanced cancers have remained problematic. Focusing on timed immune modulation may offer a fresh approach.

## References

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