Mathematical Modelling Affinity Group Meeting

Microsoft Research Cambridge, Cambridge, 7-8 June 2018

Scientific Programme

- Day 1 (7th of June 2018)
  - **10:00-10:45**
    Julia Newton-Bishop, University of Leeds.
    Myeloid shift and melanoma survival.
  - **10:45-11:30**
    Miles Carroll, Public Health England.
    Characterising naturally acquired immunity to Ebola virus: implications for vaccine licensure.
  - **11:30-12:15**
    Melissa Verheijen, Division of Infection and Immunity, University College London.
    Probing the age structure within populations of B cells.
  - **12:15-13:30**
    Lunch.
  - **13:30-14:15**
    Teresa Attenborough, Institute of Child Health, University College London.
    Immune reconstitution after cord blood transplant: the effects of subsampling.
  - **14:15-15:00**
    Jonas Mackerodt, Imperial College London.
    Mathematical models of stem cell-like memory cells (TBC).
  - **15:00-15:45**
    Jonathan Carruthers, University of Leeds.
    A novel stochastic multi-scale model of Francisella tularensis infection to predict risk of infection in a laboratory.
  - **15:45-16:15**
    Coffee break.
  - **16:15-17:00**
    Joseph R. Egan, University of Southampton.
    Mathematical modelling of cancer immunology: deterministic and stochastic considerations of receptor-ligand interactions.
  - **17:00-17:45**
    Remus Stana, University of Leeds.
    Macrophage receptor diffusion in the cytoplasm.

- Day 2 (8th of June)
  - **9:15-10:00**
    Tim Elliot, University of Southampton.
    Combining computational modelling, cellular biochemistry and structural biology to investigate peptide selector function of MHC I molecules.
  - **10:00-10:45**
    Giovanni Y Di Veroli, AstraZeneca.
    Quantitative modelling as a systematic approach for drug combination evaluation in Immuno-Oncology.
  - **10:45-11:15**
    Coffee break.
  - **11:15-12:00**
    Sergio Quezada, University College London.
    Targeting regulatory T cells for therapeutic gain: means and mechanisms.
  - **12:00-12:45**
    Ignacio Moraga, Division of cell signalling and immunology, University of Dundee.
    Boosting our "cytokinenome" with engineered synthetic cytokines.
  - **12:45-13:30**
    Lunch.
  - **13:30-14:15**
    Paul Moss, College of medical and dental sciences, University of Birmingham.
    Haemopoietic stem cell transplantation: a mathematical model of immunology.
  - **14:15-15:00**
    Marc Mansour, University College London.
    Oncogene dysregulation through somatic mutation of noncoding DNA.
  - **15:00-15:30**
    Coffee break.
  - **15:30-16:15**
    Jorge Bernardino de la Serna, UKRI STFC.
    Membrane sensing and remodelling during the immune synapse. A spatiotemporal modulation tale of lipid packing, mobility, and collective assembly.

1 Julia Newton-Bishop, University of Leeds (7th of June 2018)

Myeloid shift and melanoma survival.

Abstract The presence of a T cell infiltrate predicts a better outcome from melanoma and from checkpoint therapy for advanced disease. Understanding an absence of responses is therefore important in the hope that it will lead to better adjuvant and therapeutic treatment options. We have taken an agnostic approach to identifying peripheral blood predictors of immune infiltration at melanoma diagnosis using transcriptomes developed from blood filtered using LeukoLOCKTM filters. We adopted a bioinformatic approach to inferring immune cell signatures in the blood as described by Angelova et al. (Ref. [1]), and unpublished work shows that higher myeloid cell signatures (monocytes, eosinophils, neutrophils and MDSC) were negatively correlated with the presence of histologically detected tumour infiltrating lymphocytes (TILs). These myeloid cell scores were also positively correlated with circulating inflammatory markers. These findings are consistent with previous published work in which we reported that systemic inflammation (as inferred by association with clinical factors such as obesity, diabetes and a positive smoking history (Ref. [2]) and as measured as circulating inflammatory markers, is associated with a myeloid cell associated pro-tumourigenic environment (Ref. [3]), immunosuppressive environment. We are currently exploring immune cell signatures in 703 primary melanoma transcriptomes in order to explore associations with lack of immune responses. A paper in press (J. Clin. Invest. 2018) provides evidence that β-catenin signalling and MYC play significant roles in immune exclusion.

[1] Angelova M, Charoentong P, Hackl H, Fischer ML, Snajder R, Krogsdam AM, et al. Characterization of the immunophenotypes and antigenomes of colorectal cancers reveals distinct tumor escape mechanisms and novel targets for immunotherapy. Genome biology. 2015;16(1):64.

[2] Newton-Bishop JA, Davies JR, Latheef F, Randerson-Moor J, Chan M, Gascoyne J, et al. 25-Hydroxyvitamin D2 /D3 levels and factors associated with systemic inflammation and melanoma survival in the Leeds Melanoma Cohort. Int J Cancer. 2015;136(12):2890-9.

[3] Jewell R, Elliott F, Laye J, Nsengimana J, Davies J, Walker C, et al. The clinicopathological and gene expression patterns associated with ulceration of primary melanoma. Pigment Cell Melanoma Res. 2015;28(1):94-104.

2 Miles Carroll, Public Health England (7th of June 2018)

Characterising naturally acquired immunity to Ebola virus: implications for vaccine licensure.

Abstract TBA.

3 Melissa Verheijen, Division of Infection and Immunity, University College London (7th of June 2017)

Probing the age structure within populations of B cells.

Abstract We examined B-cell turnover throughout life using a chimeric mouse system that involves the treatment of host mice with conditioning drug busulfan. This specifically depletes hematopoeitic stem cells, leaving mature lymphocyte compartments intact. Following reconstitution with congenically labelled bone marrow, the progeny of new hematopoietic stem cells can be tracked for more than a year. Using this approach, we were able to analyse the tonic reconstitution of B-cell compartments and using hosts of varying ages, dissect the separate impacts of cell age and host age upon compartment maintenance. Interestingly, we found that the na□ve B-cell compartment is fully replaced in a relatively short time window, regardless of host age, suggesting that the B-cell compartment is both homeostatically homogeneous and highly reliant on de novo generation of B-cells for its longterm maintenance. Further investigation revealed extremely dynamic behaviour within different compartments, established by their age structure. In order to further understand the cellular mechanisms responsible for these dynamics, we considered different models for B-cell homeostasis using mathematical modelling. This analysis confirms that follicular mature B-cells behave homogeneous. All cells are lost at a fixed rate throughout life with a short lifetime. However, this life expectancy increases with host age. Similarly, germinal center B-cells are behaving homogeneous. Although these cells persist three times longer in the lymph nodes than in the spleen, modelling suggests that an extensive part of the compartment is replaced every day. Furthermore, initial model fits failed to explain the dynamics of the marginal zone B-cells, suggesting heterogeneity in this compartment.

4 Teresa Attenborough, Institute of Child Health, University College London (7th of June 2017)

Immune reconstitution after cord blood transplant: the effects of subsampling.

Abstract Spectratyping assays have generally been considered the clinical gold standard for characterising the T cell receptor (TCR) repertoire, and are widely used. However, the data that are produced are limited in several ways. Next Generation Sequencing (NGS) techniques have made it possible to capture data with much greater detail and in much higher volume. In this study we examined the process of immune reconstitution in paediatric patients who underwent cord blood transplantation (CBT) at Great Ormond Street Hospital. We used NGS to acquire the TCR repertoires from 5 control cord samples and longitudinal samples from 16 patients. The data were then analysed to quantify the TCR repertoires as the patients recovered from their transplant. A key stage of this analysis was subsampling the data in accordance with the standards in the field. Although it varied by patient, overall there were marked differences between the raw data and the subsampled data. This was evident in several aspects of the data, including the absolute diversity, and sometimes the diversity trajectory post CBT. We were able to gain a considerable insight into the immune reconstitution that follows CBT using NGS. However, we had concerns regarding the effect of subsampling on the quality of the samples. Additionally, we had reservations regarding the quantity of data that is discarded following subsampling. These concerns have motivated further interest in subsampling, and alternative methods which may retain more of the data without compromising fair comparison between samples.

5 Jonas Mackerodt, Imperial College London (7th of June 2018)

Mathematical models of stem cell-like memory cells.

Abstract One hallmark of the adaptive immune system is long-lived T cell memory. Recent studies in mice, non-human primates and human suggest that this long-lived memory is maintained by a dedicated population of cells named ?stem cell-like memory T cells? (TSCM) which are self-renewing and multipotent. We are addressing this hypothesis using an interdisciplinary approach by performing longitudinal stable isotope labelling studies in humans and applying ODE-based models. This provides unique insights into in-vivo human cell kinetics during homeostasis. Furthermore, we explore the integration of other datasets with these models, e.g., telomere length measurements, to improve the quantification of human in-vivo TSCM dynamics and develop general methods for inferring human T cell lineage topology. Preliminary results show that in comparison to modelling individuals independently, the Bayesian hierarchical modelling approach implemented yields more accurate estimates of kinetic parameters during homeostasis while also allowing for the incorporation of prior knowledge. Additionally, analysis of stable isotope and telomere length datasets of naive T cells and TSCM in healthy individuals suggests that the TSCM population is comprised of two subpopulations with a fast and slow turnover rate, respectively. The slow turning over population was found to have a lifespan and degree of self-renewal consistent with stem cells. We conclude that only a minority of TSCM are stem cell-like with dynamics that are compatible with their putative role in maintenance of T cell memory. We aim to translate these findings into the laboratory and explore the phenotypes of the putative subpopulations.

6 Jonathan Carruthers, University of Leeds (7th of June 2018)

A novel stochastic multi-scale model of Francisella tularensis infection to predict risk of infection in a laboratory.

Abstract Francisella tularensis is a highly infectious gram-negative bacterium capable of causing a debilitating disease with as few as 10 organisms, and has been classified as a category A bioterrorism agent by the Centers for Disease Control and Prevention. Previous efforts to quantify the risk to a population associated with such biological agents have used classical dose response models that focus on the probability of response but make little reference to the underlying biological mechanisms or the time until response. I will present a multi-scale stochastic model for the within-phagocyte, within-host and population level infection dynamics of Francisella tularensis. The within-phagocyte model explicitly accounts for the experimentally observed distribution of rupture times for infected phagocytes, whilst linking within-phagocyte and within-host dynamics by means of a probability mass function for the number of bacteria released in rupture events. The within-host model is used to obtain the probability of response and mean response time of an infected individual as a function of initial infection dose. A Bayesian approach is applied to parametrise both the within-phagocyte and within-host models using infection data. Finally, it is shown how these dose response probabilities at the individual level can be used for estimating the airborne propagation of Francisella tularensis in a laboratory setting at the population level, by means of a deterministic zonal ventilation model.

7 Joseph R. Egan, University of Southampton (7th of June 2018)

Mathematical modelling of cancer immunology: deterministic and stochastic considerations of receptor-ligand interactions.

Abstract A major route by which a cell interprets its environment is through the binding of signalling molecules (called ligands) to cell surface proteins (called receptors). Let R represent a receptor, L represent its associated ligand, and B represent the complex that is formed when the ligand and receptor bind together. This interaction can be described as a

reversible hetero-dimerisation reaction where the ratio of the unbinding and binding rates is known as the dissociation constant, Kd. Our presentation begins with a solution to a set of ordinary differential in a deterministic (or clockwork) framework. A stochastic (or probabilistic) simulation of the system is then implemented and its equivalence to the solution of the chemical master equation is described in the special case of the stationary distribution (i.e. when neither the mean nor the variance change with-respect-to time). We then show that if the total number of receptors, RT, or total number of ligands, LT, is much greater than the other then the stationary distribution can be approximated by a binomial distribution. In addition, if Kd is much larger than the larger of RT or LT (corresponding with a low affinity reaction and low copy numbers of B) then the binomial distribution approaches a Poisson distribution. It is known that second-order reactions combined with low copy numbers can lead to significant differences between deterministic and stochastic behaviour. Therefore, ongoing investigations are seeking to determine whether these Poisson fluctuations help to explain experimental observations at the low affinity end of the spectrum

8 Remus Stana, University of Leeds (7th of June 2018)

Macrophage receptor diffusion in the cytoplasm.

Abstract We consider simplified models in which receptors are produced in the nucleus and move under the in influence of Brownian motion until they reach the outer membrane of the cell. If the receptor encounters a peptide on the membrane it will re-enter the cytoplasm and diffuse until it is absorbed by the nucleus. Two

first passage properties of the receptor are: the mean time T from creation of a receptor to its absorption into the nucleus; and the distribution of eventual hitting points on the outer membrane. We show how these quantities can be determined explicitly for two types of geometry, namely when the nucleus and membrane are concentric and eccentric, in two dimensions. For this purpose, we derive an analytic expression for the Green's function of the Laplace equation for a domain bounded by non- concentric surfaces in two dimensions subject to absorbing outer surface and reflecting inner surface and vice versa. Making use of the Green function we derive an expression for T and compare with previous results in the literature. Furthermore, using the Green function we calculate exact formula for the distribution of eventual hitting points on the outer membrane and compare it with numerical results.

9 Tim Elliot, University of Southampton (8th of June 2018)

Combining computational modelling, cellular biochemistry and structural biology to investigate peptide selector function of MHC I molecules.

Abstract To gain a better understanding of the mechanisms of peptide selection by MHC I, we have developed a computational systems model encoding distinct mechanistic hypotheses for MHC I molecules that have different intrinsic abilities to select high affinity peptides. We fitted these models to in vivo biochemical data to infer that a conformational intermediate of MHC I is significant for peptide selection. Peptide selector function correlates with protein plasticity using molecular dynamics simulations, using site-directed mutagenesis to test predictions arising from the model directly. This approach also provided evidence that the chaperone molecule tapasin increases MHC I plasticity by a mechanism of allosteric coupling, resulting in enhanced peptide selector function. We have gone on to use the computational model to predict peptide selection from among a pool of competing peptides at different levels of intracellular abundance. The model generates a general term for the likelihood of peptide presentation that links intracellular abundance and turnover of source protein with peptide binding kinetics. The value of a mechanism-based model is clearly demonstrated by its ability to predict changes in epitope presentation under different physiological conditions including local inflammation and loss of intracellular chaperones, which both occur in the tumour microenvironment.

10 Giovanni Y Di Veroli, AstraZeneca (8th of June 2018)

Quantitative modelling as a systematic approach for drug combination evaluation in Immuno-Oncology.

Abstract Multiple strategies for eliciting and enhancing antitumor immunity are currently being evaluated. However, a more systematic approach is needed to analyze and translate such results into clinical practice (Ref. [1)]. The objective of this study was to provide predictive simulations, via a quantitative systems pharmacology (QSP) model, capable of categorizing the types of synergistic effects which may arise from IO agent combinations, across realistic baseline conditions prevailing in the tumor microenvironment (TME).

A QSP model was developed and qualified using in vivo mouse data published in the literature and from internal research. The following pharmacological modalities were calibrated: PD-L1/PD-1, CTLA-4, CXCR2 and OX40 agonism (Figure 1). Various combination scenarios were simulated for these modalities, at four baseline conditions prevailing in different syngeneic murine models.

Simulated efficacy results were highly dependent on the baseline conditions. Several combinations and monotherapies were effective only within a specific TME phenotype - these findings were in agreement with experimental data (Ref. [2]). At baselines with higher levels of MDSC, best results were obtained for a PD-L1 mAb combined with either an

OX40 agonist or a CXCR2 inhibitor, with 90% of complete responders. Anti (PD-L1 + CTLA-4) combinations showed high efficacy in Treg prevalence, but only moderate efficacy (22% complete responders) under baseline conditions of a dual (Treg + MDSC) immunosuppressive TME.

These results demonstrate a quantitative modeling framework to comparatively predict responses to IO combinations based on baseline conditions prevailing in the TME. They also reveal mechanistic interactions underlying responses in these combinations.

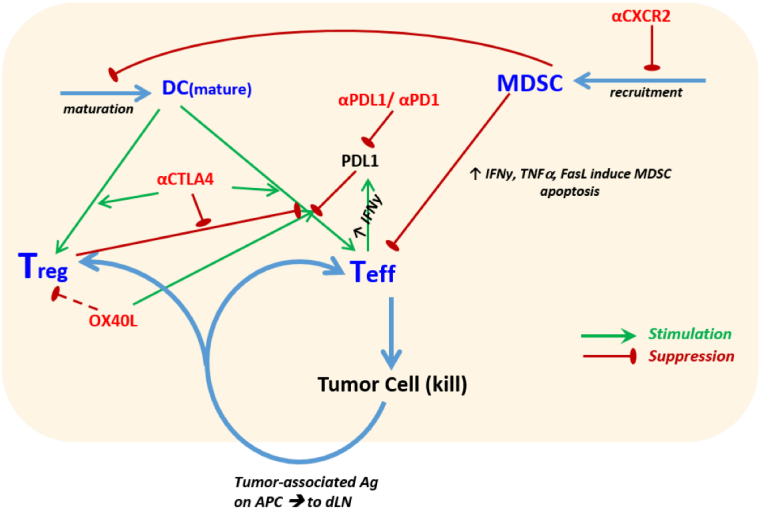

Figure 1. Schematic of the IO QSP model. *Treg:* Regulatory T lymphocytes. *Teff:* Effector T lymphocytes. *DC(mature):* Mature dendritic cells. *MDSC:* Myeloid-Derived Suppressor Cells.

[1] Melero I et al. Nat Rev Cancer 2015, 15: 457-472.
[2] Mosely S et al: Cancer Immunol Res 2016, 5: 29-41.

11 Sergio Quezada, University College London (8th of June 2017)

Targeting regulatory T cells for therapeutic gain: means and mechanisms.

Abstract Regulatory T cells have a recognised and critical role in the maintenance of immune homeostasis in mice and man. Either educated in the thymus or generated in the periphery, Tregs circulate and infiltrate tissues with the goal of fine tuning immunity and counter balance inflammatory processes to prevent autoimmunity. Despite their critical role in immune homeostasis and maintaining host integrity, their function is known to be hijacked in the context of cancer. The murine and human tumour microenvironment actively recruits regulatory T cells bearing features of activation such as upregulation of the high affinity IL2Ra (CD25) and the immune-regulatory receptor CTLA-4. In mice prophylactic and therapeutic depletion of Tregs enhances anti-tumour immunity and synergises with other immune therapies to promote tumour control. In humans the number of tumour infiltrating regulatory T cells and their spatial distribution with regards to effector T cells negatively associates with patient outcomes underscoring their negative role in anti-tumour immunity and the need for tools that tamper with their function and number. In this seminar I will discuss the role of regulatory T cells in the context of cancer, as well as old and new strategies to target this compartment in an effort to eradicate cancer.

12 Ignacio Moraga, Division of Cell Signalling and Immunology, University of Dundee (8th of June 2018)

Boosting our "cytokinenome" with engineered synthetic cytokines.

Abstract Cytokines are soluble messengers that oversee the correct functioning of the immune response. Cytokine-induced signalling is initiated via receptor dimerisation, which leads to the activation of the JAK (Janus kinase)/STAT (Signal Transducer and activator of transcription) signalling pathway. Based on the number of cytokine receptor encoded in our genome, the number of cytokines found in humans only represents a small fraction of the more than 1600 ligands that in theory the cytokine system could accommodate. Using a large matrix of chimeric cytokine receptors we show that non-natural cytokine receptors pairs are compatible with signalling, highlighting the large functional plasticity of

the cytokine-cytokine receptor system. Additionally, we have engineered a series of new synthetic cytokines, based on IFN, IL-4 and IL-2 cytokines. These synthekines assemble IL-2Rb/IL-4R or IL-4R/IFNAR2 receptor heterodimers that do not occur naturally and trigger signalling and functional responses distinct from those activated by IL-2, IL-4, and IFN. Synthekines represent a new family of ligands that could potentially surmount the limitation that natural cytokines face when translated to the clinic.

13 Paul Moss, College of Medical and Dental Sciences, University of Birmingham (8th of June 2018)

Haemopoietic stem cell transplantation: a mathematical model of immunology.

Abstract Stem cell transplantation (SCT; previously known as bone marrow transplantation) is an effective treatment for many patients with leukaemia. The procedure involves administration of chemotherapy to the patient followed by infusion of blood ?stem cells? which are taken from a donor. These cells then reconstitute the entire blood and immune systems in the patient.

A fascinating feature of SCT is that it is now clear that the immune system of the donor plays an important role in killing residual leukaemia cells in the patient and this "graft versus leukaemia" effect (GVL) is very important in controlling disease. However, although many patients are cured of their disease, SCT has many complications and only around 60% of patients survive at 5 year follow up.

We are particularly interested in the first two weeks following transplant, a period during which the immune response against the patient is developed. We find that the number of different subpopulations of immune cells is very important in determining the ultimate outcome of the patient. This is revealing mechanisms that may play an important role in GVL. Our ambition is to be able to predict, and appropriately manage, the outcome for patients based on information available at day 14. Working with colleagues in the Department of Mathematics, we are using novel statistical approaches to achieve this ambition.

14 Marc Mansour, University College London (8th of June 2018)

Oncogene dysregulation through somatic mutation of noncoding DNA.

Abstract Identification of the genes specifically involved in chromosomal translocations in different cancers has paved the way for modern day diagnostics, prognostication, minimal residual disease analysis, disease modeling in vivo, and now, as is the case for CML, highly efficacious targeted therapy. Despite the appreciation that aberrant expression of one or more key oncogenes is a feature of most cancers, many cancers lack cytogenetically detectable chromosomal lesions to explain underlying oncogene dysregulation. T-cell acute lymphoblastic leukaemia (T-ALL) provides an excellent example, given over one-third of cases have no known driver lesion by standard cytogenetic analysis. We hypothesized that these cases were only normal at the resolution of cytogenetic analysis, instead being driven by somatically acquired gain-of-function mutations at noncoding sites that create neomorphic enhancers and promoters. In support of this, we recently described how small indels in a noncoding site create a neomorphic enhancer that loops to, and drives overexpression of the prototypic T-ALL oncogene, TAL1. Here, the somatically acquired indels create de novo binding sites for the transcription factor MYB, which binds to its new site together with its partner CBP. CBP is a histone acetyltransferase that acetylates H3K27, leading to aberrant enhancer formation that drives monoallelic TAL1 expression.

Allelic skewing of gain-of-function mutations is readily detectable in ChIP-seq reads, forming the basis of a discovery tool to identify novel noncoding driver mutations. Utilizing this method, we have identified somatic mutations at a noncoding site that create an enhancer to drive LMO2 expression in T-ALL. Mutation hotspots do not occur at random regions of the genome, instead clustering at loci that are involved in enhancer or promoter formation during specification of other cell types. Identification of gain-of-function noncoding mutations that have been selected for during tumorigenesis in vivo, thus offers important insights into the optimal DNA syntax required for nucleation of such multi-protein transcription factor complexes, as well as having the potential to identify novel cancer drivers.

15 Jorge Bernardino de la Serna, UKRI STFC (8th of June 2018)

Membrane sensing and remodelling during the immune synapse. A spatiotemporal modulation tale of lipid packing, mobility, and collective assembly.

Abstract Lymphocyte T cells are responsible for cell-mediated adaptive immune responses, involving transient interactions of the T-cell receptor (TCR) with peptides presented by MHC proteins. A productive interaction triggers the T cell signalling forming the immunological synapse (IS). Initially, Lck, a membrane-anchored tyrosine kinase, phosphorylate the TCR, ultimately producing basal plasma membrane microclusters and calcium release. For this purpose, the preferred imaging method to unravel nanoclustering at surface-membrane close contact zones during in-vitro T cell activation is TIRF-based super-resolution imaging (i.e., dSTORM and PALM). Classically, clustering characterisation of

the resting (non-activated) state relied in observing T cell onto surfaces coated with Poly-L-lysine; whereas T cells, onto functionalised surfaces coated with anti-CD3, and -CD28 antibodies, resembled the T cell activation during the IS. In the last years, some super-resolution studies suggested protein nanoclustering already at resting state, which contradicts the consensus picture of protein aggregation upon activation. Recently, results from T cells in controlled suspension (immersed in a hydrogel gradient) and later on following its activation highlighted that unnatural cell membrane interactions might hinder our understanding of early T cell activation and the subsequent IS. Whereas these studies focused in the protein clustering and its relation to cortical actin dynamics, they overlooked at the role of the lipids membranes in the early activation and during the IS. Typically, protein clustering is assumed to be coordinated with a higher lateral lipid packing at the membranes. We aim at gaining some knowledge on how lipids would behave in these highly dense localised protein aggregation conditions. For this purpose, to better understand how T cell membranes sense and remodel during the immune synapse, we employ fluorescence imaging correlation spectroscopy based methods and reveal the membrane lipid spatiotemporal localisation, diffusion, and collective motion at the plasma membrane.