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TUMORS AND IMMUNE SYTEMS : FROM THEORY TO THERAPY

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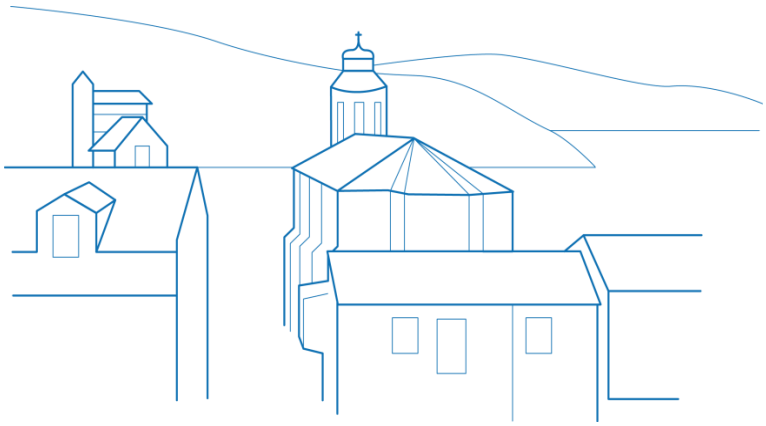

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Tumors and Immune Systems: From Theory to Therapy

April 15-19 2019

	Monday 15	Tuesday 16	Wednesday 17	Thursday 18	Friday 19	Saturday
9:00 – 9:40	Greenbaum	De Carvalho	H Levine	Buettner	Walczak	departure
9:45 – 10:25	Luksza	Beyer	Chakraborty	Balachandran	Merghoub	
10:30 – 11:00	Coffee	Coffee	Coffee	Coffee	Coffee	
11:00 – 11:40	McGranahan	Domany	Cocco	Thomas	Lässig	
11:45 – 12:05	Elhanati	Vabret	Minervina	Bussemaker	Sulc	
12:05 – 12:25	Cvijovic	Mayer	Soumelis	Kondratova		
12:30 – 14:15	Lunch	Lunch	Lunch	Lunch	Lunch	
14:15 – 14:35	Berg	poster session	Free time	Bruscolini	Discussion	
14:35 – 14:55	George			Körber		
15:00 – 15:20	Dupic	Coffee		Lepoutre	Coffee	
15:20 – 15:40	Sharonov	poster session		Coffee	Free time	
15:40 – 16:00	Coffee			Free time		
16:00 – 19:00	Free time					
	Welcome cocktail			BBQ		

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IRN Predictability Adaptation Navigation

Tumors and Immune Systems: From Theory to Therapy

Cargèse, April 15-19, 2019

Benjamin Greenbaum, Michael Lässig, Rémi Monasson, Thierry Mora

Titles and abstracts

Luis Aparicio

Universality of Random Matrix Theory to study single-cell multi-omics systems.

The development of single-cell technologies provides the opportunity to identify new cellular states and reconstruct novel cell-to-cell relationships. Applications range from understanding the transcriptional and epigenetic processes involved in metazoan development to characterizing distinct cells types in heterogeneous populations like cancers or immune cells. However, analysis of the data is impeded by its unknown intrinsic biological and technical variability together with its sparseness; these factors complicate the identification of true biological signals amidst artifact and noise. Here we show that, across technologies, roughly 95% of the eigenvalues derived from each single-cell data set can be described by universal distributions predicted by Random Matrix Theory. Interestingly, 5% of the spectrum shows deviations from these distributions and present a phenomenon known as eigenvector localization, where information tightly concentrates in groups of cells. Some of the localized eigenvectors reflect underlying biological signal, and some are simply a consequence of the sparsity of single cell data; roughly 3% is artifactual. Based on the universal distributions and a technique for detecting sparsity induced localization, we present a strategy to identify the residual 2% of directions that encode biological information and thereby denoise single-cell data. We demonstrate the effectiveness of this approach by comparing with standard single-cell data analysis techniques in a variety of examples with marked cell populations.

Vinod Balachandran

Learning rules of immune recognition from long-term pancreatic cancer survivors

Johannes Berg

Resistance to targeted cancer therapy: lessons from large cell cultures

Targeted therapy to cancer acts on the molecular abnormalities specific to a particular tumor. This specificity turns out to be a double-edged sword: For a given targeted therapy, there can be many mutations which make a cancer cell resistant to therapy, and typically-sized tumor can contain many different types of resistant mutants. As a result, response to targeted therapy is almost always short-lived: therapy-susceptible cells are eradicated, whereas resistant cells continue to grow under therapy. To prevent the rapid emergence of resistance, one needs to anticipate the resistance mechanisms present in a tumor at the onset of therapy. We study the spectrum of resistant cells in a well-known cell-line model of lung cancer and identify drug combinations covering the full spectrum of the resistance mutations present.

We use an iterative scheme of artificial evolution to amplify and isolate different resistance mechanisms to targeted therapy from large cell cultures. As a proof of concept, we apply our scheme to PC-9 cells, a non-small cell human lung cancer cell line with an activating mutation in EGFR. From large populations of PC-9 cells, we iteratively isolate cells resistant to EGFR inhibition. We find that mutants with distinct resistance mechanisms have distinct growth rates, which influence both their frequencies prior to therapy and the particular mechanism which eventually takes over the population. The mechanisms we find include the well-known gatekeeper-mutation T790M, amplification of the MET-ligand HGF, as well as a mechanism based on mTOR. A drug combination targeting all these mechanisms prevents the expansion of resistant cells. This combination also turns out to inhibit the growth of drug-tolerant cells, which can otherwise act as a reservoir for further resistance mutations. Our iterative scheme offers a generic approach to explore the spectrum for resistance mutations and can be applied to cell lines driven by different molecular mechanisms."

Andreas Beyer

Integrating molecular layers for understanding cancer phenotypes

Barbara Bravi

A statistical physics approach to immune responses in pancreatic cancer

Pierpaolo Bruscolini

Humanization of Antibodies using a Statistical Inference Approach

Antibody humanization is a key step in the preclinical phase of the development of therapeutic antibodies, when originally developed and tested in non-human models. The standard technique of Complementarity-Determining Regions (CDR) grafting into human Framework Regions of germline sequences has some important drawbacks, in that the resulting sequences often need further back-mutations to ensure functionality and/or stability. Inspired by Baldassi et al., PloS one 9, e92721 (2014), we propose a new method to characterize the statistical distribution of the sequences of the variable regions of human antibodies, that takes into account phenotypical correlations between pairs of residues, both within and between chains. We define a “humanness score” of a sequence, comparing its performance in distinguishing human from murine sequences, with that of some alternative scores in the literature. We also compare the score with the experimental immunogenicity of clinically used antibodies. Finally, we use the humanness score as an optimization function and perform a search in the sequence space, starting from different murine sequences and keeping the CDR regions unchanged. Our results show that our humanness score outperforms other methods in sequence classification, and the optimization protocol is able to generate humanized sequences that are recognized as human by standard homology modeling tools.

Reinhard Buettner

Molecular Lung Pathology: Selecting Patients for Immune Therapies versus Targeted Therapies

Approximately 20-25% of cancer mortality results from lung cancer. Traditionally, all patients in advanced tumor stages received combined chemotherapies with a very poor survival of less than 12 months. Indepth molecular investigations revealed a large number of oncogenic driver mutations, which render these tumors vulnerable to small molecule inhibitors. More recently, immune checkpoint inhibitors were introduced successfully for patients not eligible for targeted therapies. Important parameters predicting responses to immune therapies include expression of immune checkpoints, tumor mutational burden and activation signatures of cytotoxic T-cell infiltrates.
The aim of this seminar is to outline the key molecular parameters used for selection of effective therapies.

Harmen Bussemaker

From millions of DNA reads to mechanistic insight into biomolecular recognition

In recent years, in vitro profiling of protein-DNA interactions using massively parallel DNA sequencing (such as HT-SELEX, SELEX-seq, or SMILE-seq) has been performed for hundreds of transcription factors (TFs). Our lab has developed hierarchical maximum likelihood methods that embed a feature-based biophysical model of binding free energy within a statistical model of the high-throughput assay, and demonstrated that the resulting

models yield unprecedented mechanistic insight into transcription factor function. No Read Left Behind (NRLB; Rastogi et al., PNAS, 2018), allows us to quantify the binding specificity of transcription factor complexes almost perfectly over a >100-fold affinity range and an unlimited binding site footprint. When ultra-low-affinity (i.e., 300-fold weaker than the best site in the genome) homeodomain (Hox) binding sites in Drosophila enhancers are mutated, NRLB accurately predicts the effect on reporter expression in embryos. In this presentation, I will review NRLB and describe ongoing efforts in our lab to adapt it to the problem of receptor-epitope interaction specificity in the context of the adaptive immune system based on deep sequencing of receptor repertoires.

Arup Chakraborty

TBA

Victor Chardès

Optimal response to pathogen evolution in immune repertoires

In order to target threatening pathogens, the adaptive immune system performs a continuous reorganisation of its immune cell repertoire constrained on the antigenic load. This emergent self-organised repertoire results from a variety of biological processes such as affinity maturation or thymic selection. Previous work formulated a theory predicting immune repertoire organisation, assuming that it results from an optimization problem with biological constraints. Here we extend this formalism to a co-evolutionary setting, where we explicitely consider the effects of a continuously mutating viral population and immune cell maturation processes on the dynamics of immune repertoire adaptation.

Andrew Chen

Genomic and immunological study of response to anti-PD-1 immunotherapy in glioblastoma

Immune checkpoint inhibitors have been successful against many cancers; however, their efficacy has been uncommon and unpredictable in glioblastomas (GBM), where <10% of patients show long-term responses. To understand the molecular determinants of immunotherapeutic response in GBM, we have longitudinally profiled 66 patients, including 17 long-term responders, during standard therapy and after treatment with PD-1 inhibitors. We found a significant enrichment of PTEN mutations associated with immunosuppressive expression signatures in non-responders, and an enrichment of MAPK pathway alterations

(PTPN11, BRAF) in responders. Responsive tumors were also associated with branched patterns of evolution from the elimination of neoepitopes, as well as differences in T cell clonal diversity and tumor microenvironment profiles. We shows that clinical response to anti-PD-1 immunotherapy in GBM is associated with specific molecular alterations, immune expression signatures, and immune infiltration that reflect the tumor's clonal evolution during treatment. REPLACES While immunotherapy has demonstrated success in melanoma, a deeper understanding of the heterogeneous tumor microenvironment is needed for stratifying patients for treatment. Technologies such as quantitative multiplex immunofluorescence imaging and transcriptomic profiling both have the potential to provide such insights. However, the connection between these modalities and their impact on prognosis is not well-understood. Here, we investigate the link between the microenvironmental composition of immune cells, such as cytotoxic T lymphocytes and macrophages, with observed transcriptomic signatures. Furthermore, we uncover spatial correlations in cellular positioning, which supports a mechanistic basis underlying these relationships. Understanding these connections between the imaging and the genetics of melanoma will bring us closer to forming effective clinical biomarkers for evaluating immunotherapies.

Simona Cocco

Finding immunogenic nucleotidic motifs in viruses and tumors : Statistical Physics approach

Ivana Cvijovic

High-resolution lineage tracking reveals traveling wave of adaptation in laboratory yeast

In rapidly adapting asexual populations, including many microbial pathogens and viruses, numerous mutant lineages often compete simultaneously for dominance within the population. These complex evolutionary dynamics determine the outcomes of adaptation, but they have been difficult to observe directly. While earlier studies used whole-genome sequencing to follow molecular adaptation, these methods have very limited frequency resolution in microbial populations. Here, we introduce a novel renewable barcoding system to observe evolutionary dynamics at high resolution in laboratory budding yeast. We find nested patterns of interference and hitchhiking even at low frequencies. These events are driven by the continuous appearance of new mutations that modify the fates of existing lineages before they reach substantial frequencies. We observe how the distribution of fitness within the population changes over time, finding a "traveling wave" of adaptation that has been predicted by theory. We show that the dynamics of clonal competition create a dynamical rich-get-richer effect: fitness advantages acquired early in evolution drive clonal expansions, which increase the chances of acquiring future mutations. However, less-fit lineages also routinely leapfrog over strains of higher fitness. Our results demonstrate that

this combination of factors, which is not accounted for in any existing model of evolutionary dynamics, is critical in determining the rate, predictability, and molecular basis of adaptation.

Daniel De Carvahlo

TBA

Carino DIAS GURJAO

Tumor mutational load as a predictor of response to immunotherapy

Eytan Domany

Pathway-based personalized analysis of breast cancer

I will present a “systems approach” to analysis of high throughput large cancer datasets. The basic idea is to make use of existing knowledge, taking the golden path between “ignorance-based” machine learning approaches and the “all details are essential” view of many biologists. This philosophy [1] has been implemented in Pathifier – an algorithm that infers pathway deregulation scores for each individual tumor sample, on the basis of expression data [2]. The algorithm transforms gene level information into pathway level information, generating a compact and biologically relevant representation of each sample. The method avoids the pitfall referred to as “the curse of dimensionality” – for every step of the analysis, the number of data points exceeds significantly the number of dimensions in which the points are embedded.

We applied the method to a very extensive dataset on breast cancer [3], and discovered that a cluster of immune system related pathways divides an enigmatic subgroup of breast cancer patients to two groups – of low/high immune involvement. This stratification has clinical significance (in terms of outcome) and a clear biological interpretation (level of tumor infiltrating T cells). These observations led us on a roller coaster ride that seems to identify the presence/absence of T cells as predictive of response to some “classical” chemotherapies. Some very preliminary reflections on possible mechanisms for the role of immune cells in assisting cell death induced by chemotherapy may be mentioned briefly.

Our findings can be easily incorporated into the current decision making pipeline on

treatment, and hence may have clinical implications for 2-3% of women with breast cancer (e.g. spare them from receiving ineffective chemotherapy).

[1] Using High-Throughput Transcriptomic Data for Prognosis: A Critical Overview and Perspectives. Eytan Domany, Cancer Research 74, 4612 (2014).
[2] Pathway-based personalized analysis of cancer. Yotam Drier, Michal Sheffer, and Eytan Domany, PNAS 110, 6388 (2013)
[3] Pathway-based personalized analysis of breast cancer data. Anna Livshits et al, Molecular Oncology 9, 1471 (2015).

Thomas Dupic

Genesis of the αβ T-cell receptor

T-cell receptors (TCR) are molecules expressed at the surface of T-cells, and composed of two protein chains alpha and beta. They are responsible for recognizing antigens, and their diversity underlies the adaptability of the immune system. This diversity is created during a process called
V(D)J recombination where the two genes coding for the protein chains are randomly arranged. This recombination can happen independently on the two homologous chromosomes hence a cell may contain two recombined genes for the same chain.
Until recently most experiments and analyses focused on only one of the two chains at a time. We used data obtained through high-throughput sequencing method to simultaneously characterize the distribution of both chains in a repertoire. The correlations between sequences sharing a cell offer
new insight into the generation process. In particular, the correlation between alpha sequences sharing a cell allows us to quantify the impact of the rescue mechanism. We show that the alpha and beta chain recombine independently, and that many cells express two alpha chains. Our statistical analysis also permits us to revisit the questions of the diversity and overlap of TCR repertoires at the level of alpha-beta pairs.

Yuval Elhanati

Statistical investigations into the T cells response to cancer

One of the properties of cancer cells is their high mutation rate, which can create many neo-antigens - targets for the immune system. The adaptive immune system maintains a large diversity of T-cells with different surface receptors, which can bind to a neo-antigen in the cancer cells and mobilize the immune system against them. Following antigen recognition, activated T cells undergo proliferation dynamics, depending on the clinical situation and the tissue involved. Specifically, it is conjectured that a good immune response to cancer will involve expansion of tumor specific T cells within the tumor micro-environment.

Next Generation Sequencing enables us to follow the dynamics of the T-cells ensembles in blood and tissue samples, which suggest that response dynamics are convoluted and involve a large ensemble of T cells, not all of them fully reactive. To overcome the complex response dynamics and sample noise, we analyze such samples using statistical models, based on sequence and count data, that can infer and characterize the distribution of T-cells. Based on these distribution, we compare the immune system from different time points, patients and organs, and analyze the response to the cancer. Eventually we can find candidate reactive T cells and immune system characteristics related to the cancer that could predict the outcome of treatments.

Ioan Filip

HLA typing from RNAseq and applications to cancer

The human leukocyte antigen (HLA) complex, necessary for antigen presentation, regulates both innate and adaptive immune responses. The HLA locus therefore plays a critical role in a plethora of diseases, particularly in cancer and response to therapy. While recent improvements in the quality and accessibility of next-generation sequencing have made HLA typing from standard short-read data practical, this task remains challenging given the high level of polymorphism and homology between the HLA genes.
Here, we present arcasHLA: a fast and accurate in silico method to infer HLA genotypes from RNA sequencing data. Our tool outperforms the established HLA typing tools on gold-standard benchmark datasets, with 100% accuracy at two-field resolution for HLA class I genes, and over 99.7% accuracy for HLA class II. As an application, we present some work in progress aiming to detect HLA allelic expression imbalance in several cancer datasets. In collaboration with Rose Orenbuch, Itsik Pe'er and Raul Rabadan (https://www.biorxiv.org/content/10.1101/479824v1 and https://github.com/RabadanLab/arcasHLA).

Jason George

Stochastic tumor-immune co-evolution quantifies cancer incidence and immunotherapy efficacy

Recent advances in immunotherapy have revolutionized modern cancer treatment. While encouraging, robust therapies that lead to durable remission outcomes still remain a challenge in curing many malignancies as cancer cells may acquire clones that effectively evade the immune system. Optimizing this treatment has proven difficult owing to the complexity of the tumor-immune system interaction. In extreme cases, hematopoietic stem cell recipients enlist an entire donor-derived allogeneic T-cell repertoire with the hope of eliciting an anti-tumor effect. Perhaps most importantly, the adaptive nature of the immune system uniquely enables this treatment approach to co-evolve alongside an evasive threat. However, this process is poorly quantified and thus merits further study in order to maximally benefit cancer patients. Here, we apply elements of stochastic process theory to develop a

theoretical framework which quantifies the dynamics between a growing collection of cancer cells capable of acquiring evasive clones and a T-cell repertoire that may eventually recognize the evading populations. We show that our model is consistent with known tumor evolutionary data, and we relate differences in branched vs. clonal evolution to the stringency of immuno-surveillance present during early cancer progression. We estimate the fraction of all tumor initiating events that ultimately result in observed incidence, and partition cancer sub-types based on predicted immune enhancement or compromise. In conclusion, our co-evolutionary model recapitulates empirical observations and offers a theoretical framework for quantifiable predictions to further improve cancer immunotherapy.

Benjamin Greenbaum

Overview and introductory remarks

Adrien Jolly

Thymocytes reprogram in the face of progenitor shortage

In the mouse, new T cells are generated in the thymus during the lifetime of the animal. Progenitors from the bone marrow settle in the thymic cortex and there begin a differentiation process, including sequential recombination of the beta and alpha chains of the T cell receptor (TCR) and clonal selection based on the affinity of the TCR for MHC self-peptide complexes presented by thymic epithelial cells. Under physiological conditions, the thymocyte population is replaced within weeks by influx from bone-marrow-derived progenitor cells. However, thymi become self-sufficient and continue to produce new T cells for months when transplanted into recipients that do not produce competent BM progenitors. To explain this phenomenon, we first developed a mathematical model for the development of early thymocytes (double-negative, DN, for CD4 and CD8) that assumes competition of the descendants of incoming progenitors with resident DN thymocytes for proliferation and/or survival signals. The model predicts that loss of competition by abrogating progenitor input enhances proliferation and/or survival of resident thymocytes. Subsequently, we observed experimentally that a specific subpopulation of thymocytes, the DN3, exhibits dramatically increased proliferation in the autonomous context whereas no such increase was observed in the downstream compartments. Using mathematical modeling of cell cycle and cell differentiation, as well as joined single-cell RNAseq and TCR sequencing, we have uncovered complex changes in DN3 phenotype involving both increased proliferation and altered differentiation in response to preTCR signaling, the key stimulus at this stage of development. Altogether, our results point to the reprogramming of the DN3 compartment that sustains thymic output in the face of abrogated progenitor cell input from the bone marrow.

MARIA KONDRATOVA

ATLAS OF CANCER SIGNLLING NETWORK FOR ANALYSIS OF HETEROGENEITY AND FUNCTIONAL POLARIZATION OF CELLS IN TUMOR MICROENVIRONMENT FROM SINGLE-CELL DATA

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Keywords
Cancer, molecular network reconstruction, network visualization, data analysis, innate immune response, tumor microenvironment, single-cell data.

Atlas of Cancer Signaling Network (ACSN) is a pathway database and an interactive comprehensive network map of cancer-related molecular mechanisms (https://acsn.curie.fr/). The resource includes tools for map navigation, visualization and analysis of molecular data in the context of signaling network maps. The database also includes several networks describing cell-type specific molecular interactions various cells present in tumor microenvironment (TME).

Tumor microenvironment plays important and sometimes, opposite roles in tumor evolution. Interactions both immune and non-immune cells are important for effective anti-tumor response. We applied our networks to analyse non-immune (cancer associated fibroblasts (CAF)) and immune (macrophages (Mph) and natural killers (NK)) components of this multicellular system.

CAF map (681 objects, 585 reactions) is a network depicting most significant pathways involved in fibroblast activation in cancer. The CAF map has a modular structure representing all main functions of CAFs in a tumor. Three modules represent CAF interactions with extracellular matrix (“Integrin signalling pathways”, “Motility"" and ""Matrix regulation”) Two modules represent participation of CAFs in tumor growth growth (“Growth factors signaling pathways”, and “Growth factor production”) and two modules contain interactions of fibroblasts with immune system cells (“Inflammatory signalling pathways”, ""Chemokine-cytokine production”). “Metabolic” module includes the main pathways involved in metabolic reprogramming of CAFs and ROS production. Modules “Markers of fibroblast activation” and molecules preventing fibroblast activation (“Antitumor”) complement the network.

Meta-map of innate immune response in cancer contains 1476 objects and 1085 reactions. We first constructed cell-type specific signalling maps of macrophages, dendritic cells, myeloid-derived suppressor cells, natural killers, neutrophils and mast cells. These maps integrated together and updated by interactions and crosstalks between them and the map of tumor cell, gave rise to a seamless comprehensive meta-map of innate immune response in cancer, demonstrating signalling responsible for anti- and pro-tumour activities of innate immunity system as a whole. It is a ‘geographical-like’ hierarchically-organized meta-map

with functional ‘zones’: namely, signalling mechanisms contributing to anti-tumor or pro-tumor immune phenotypes.

In particular, CAF and Innate immune map of ACSN were used to interpret single cell RNA-Seq data for fibroblasts and immune cells from tumor samples. The analysis demonstrated the existence of sub-populations within each cell type that possess different polarized anti- or pro-tumor status.
Analysis and interpretation of expression patterns from breast cancer CAFs samples in the context of the network map helped to characterize different CAF subsets with distinct molecular properties and levels of activation. The enrichment analysis of transcriptome and gene sets from functional modules of CAF map highlighted that CAF-S1 subset exhibits a high expression of immune signatures, including cytokines production and modulation of regulatory T lymphocytes (Tregs), promoting an immunosuppressive microenvironment. Whereas, CAF-S4 subset is exhibiting matrix regulation and motility mechanisms, indicating that CAF-S4 subset most probably modulates properties of extracellular matrix and facilitates tumor invasion.

The macrophage population in melanoma consists of two types of cells; one is characterized by anti-tumor activity, whereas the second one is oriented towards pro-tumoractivity. Activated subset of NK cells in melanoma were characterized by induction of LFA1, CR3 and FcGR2 pathways involved in triggering tumor-killing signaling, indicating anti-tumor polarization status of NK in the studied sub-sets of cells.

The ACSN is a useful resource for computing network-based molecular signatures of immune cells polarization. These signatures will help to characterize the overall status of the signalling dictating pro-tumor and anti-tumor states of tumor microenvironment (TME) in cell lines and tumor samples. It will also help to stratify cancer patients according to the status of the TME and potentially predict patient survival and response to immunotherapies.

Verena Körber

Evolutionary trajectories of de novo glioblastomas reveal a common path of early tumorigenesis instigated years ahead of initial diagnosis

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We studied how intratumoral genetic heterogeneity shapes tumor growth and therapy response for de novo glioblastoma, a rapidly regrowing
tumor. To this end, we inferred the evolutionary trajectories of matched pairs of primary/relapsed tumors based on deep whole-genome-sequencing. Combined analysis of tumor phylogenies with population dynamics models of tumor growth suggests both a distant origin of de novo glioblastoma, up to seven years before diagnosis, and a common path of early tumorigenesis, with one or more of three events (chromosome 7 gain, 9p loss or 10 loss) at tumor initiation. TERT promoter mutations often occurred later as a prerequisite for rapid growth. In contrast to this common early path, relapsed tumors acquired no stereotypical pattern of mutations and typically regrew from oligoclonal origins, suggesting sparse selective pressure by therapeutic measures.

Michael Lässig

Eco-evolutionary control of pathogen populations

Thomas Lepoutre

Impact of the immune system on chronic myeloid leukemia

Chronic myeloid leukemia is a blood cancer for which there exists a very efficient targeted therapy (Tyrosine Kinase Inhibitors). While this has revolutionnized the long term prognosis of treated patients, the next question is the possibility of stopping the treatment and thereby entering so called Treatment Free Remission (TFR). We present recent results on the mathematical modelling of chronic myeloid leukemia. Describing the interaction between chronic myeloid leukemia and autologous immune response, we propose an interpretation of treatment free remission as a stability property. The interpretation is then a control of the disease by the immune system rather than an eradication.

Herbie Levine

Towards a Theory of Tumor Neoantigen Recognition

Marta Luksza

TBA

Cosimo Lupo

V-gene insertions and deletions during the affinity maturation process in BCR repertoires

The ability of the immune system to recognize and kill a huge range of external pathogens is ensured by a high diversity in the binding sites of membrane Receptors in B-Cell lymphocytes (BCR). The resulting repertoire of BCRs is updated and increased via a 2-step stochastic process for the creation (recombination) and the evolution (affinity maturation) of each nucleotide sequence encoding the receptors. The common picture of the recombination process involves a random choice of the genes from the germline DNA, plus some nucleotide deletions and insertions (briefly, indels) at the junctions of such genes. Instead, the affinity maturation of the sequence involves some context- and position-dependent point mutations, namely the exchange of some nucleotide bases. Our analysis focuses on the possibility of experiencing indels not just at the junctions between the germline genes in the recombination process, but directly in the bulk of the most variable (V) gene in the chain during the affinity maturation stage, further enhancing the variability of the repertoire. These indels appear prominently in the BCR of both healthy people and HIV-responding broadly neutralising antibodies. We evaluate the probability of such indels, with the aim of developing a likelihood-based approach for their inference from real data and for the generation of more reliable synthetic sequences.

Andreas Mayer

A statistical ensemble approach to immune discrimination

The immune system needs to distinguish molecular signatures of pathogens from those found in the organisms' own proteins. A naive, but universal way to discriminate is to whitelist everything that should not elicit a reaction. Can the immune system do better? To begin to answer this question we characterize the self and pathogen proteomes as statistical ensembles. Probabilistic models reveal how both universal and phyla-specific constraints on protein evolution shape the statistics of the proteomes. The models furthermore allow us to quantify to what extent the ensembles differ systematically. We analyze whether and how these differences might be used for efficient immune defense. Finally, we compare predictions to what is known about epitopes recognized by the immune system.

Nicholas McGranahan

TBA

Taha Merghoub

How to overcome resistance to immune checkpoint blockade

Vanessa Mhanna

Deciphering the TCR repertoire diversity of regulatory and effector T cell subsets in healthy mice from NGS data

FoxP3+ regulatory T cells (Tregs) play a critical role in immune homeostasis and in controlling immune responses to self, tumors and pathogens. We previously described two sub-populations of murine thymus-derived Tregs based on their activation status: CD44lowCD62Lhigh naïve Tregs (nTregs) with a low turnover, and CD44high CD62Llow activated/memory (amTregs) with a very rapid turnover and which are enriched in organ-draining lymph nodes. To study the repertoire dynamics of these subsets, we launched a descriptive Treg repertoire cartography in the thymus and secondary lymph nodes of C57BL/6 (B6) mice, a healthy physiological model. A first study of splenic Treg TRB repertoire in young mice revealed a reduced diversity and more clonotype expansions in amTregs compared to nTregs and the conventional effector T cells, as well as differences in TRBV expression between the three populations. In addition, amTregs shared a higher proportion of their repertoire with nTregs than Teffs. This suggests that nTregs could be in part precursors to the amTreg population. Finally, the study of the structural diversity of the most expanded TRB sequence revealed a convergence in the nTreg and Teff sequences which formed dense similarity clusters, while amTreg TRBs were less connected. This accentuates the hypothesis of the presence of biases in the TCR recombination and/or selection processes, which is reshaped in the periphery by antigen experiencing. We propose here an integrated workflow dissecting immune repertoire topology that can be transposed to various biological questions. Importantly, we explore the impact of data normalization on our results in order to take advantage of the huge amount of Adaptive Immune Receptor Repertoire Sequencing datasets.

Anastasia Minervina

TCR repertoire sequencing reveals diverse individual trajectories of clonotypes responding to yellow fever vaccination

Yellow fever vaccination is a well established model of acute viral infection in human. Primary immunization elicits strong T-cell response and formation of long-lived memory. However, little data exists on T-cell response to revaccination. We applied deep TCR-profiling to track T-cell clones after yellow fever vaccination and revaccination. We isolated PBMCs, CD4+, CD8+, memory and MHC-dextramer positive subpopulations from first-time vaccinee and revaccinated donor on several timepoints before and after YFV17D immunization. TCR repertoires were reconstructed and expanded TCR were identified using

edgeR. We identified 1580 expanded clonotypes in first-time vaccinee and 204 in revaccinated donor. YFV17D-specificity was confirmed by sequencing of MHC-dextramer positive subpopulation. In first-time vaccinee fraction of responded clones sharply peaked (6.1%) at day 15 after immunization. However, in revaccinated donor fraction of responded clones reached 0.4% by day 5 and slightly increased on days 10 and 15. Analysis of individual clones dynamics showed presence of two distinct groups of clones in revaccinated donor. T-cell response to booster YFV17D vaccination, even 30 years after the first, differs from response to primary immunization both by intensity and dynamics. We detected early expansion of some T-cells in response to revaccination and delayed response of putatively naive T-cells.

Marco Molari

Modelling affinity maturation under different immunization schemes: the role of antigen dosage

No medical procedure has been more successful in saving lives than vaccination. The core mechanism underlying vaccination is affinity maturation (AM), a darwinian evolution process whose outcome is a population of antibodies with high affinity for the administered pathogen. Recently, effort has been put into devising optimal immunization strategies by controlling the antigen (Ag) dosage and administration schedule. Following these approaches we introduce an analytical model for AM with which we aim to study the effect of Ag dosage on the outcome of immunization. Through the solution of an eigenvalue equation we find that our model presents different asymptotic phases according to the Ag concentration. In particular one phase at intermediate concentration appears to be optimal for developing high affinity antibodies. By including the dynamics of Ag decay and consumption, the model is able to reproduce data obtained by immunizing mice with different dosages and delay between injections.

Thierry Mora

Design principles of optimal immune systems

Carlos Olivares

IGoR's web user interface

The adaptive immune system is a highly specialized system and plays a crucial role to defend a vertebrate against a large number of pathogens by triggering specific mechanism to recognize them.
The most important mechanisms to achieve a big repertoire of antibodies and receptors are the somatic V(D)J recombination and hypermutation. These

two processes are driven by stochastic events, hence a statistical approach should use to analyze how the immune repertoire is generated. In that sense, IGoR is a software developed to learn V(D)J statistics from datasets of B or T cell receptor sequences, find the most probable scenarios and generate sequences based in the inferred models. Therefore, we develop a web interface for IGoR to get graphical representations of the inferred models, evaluate sequence statistics and generate synthetic sequences from an inferred model in an easier and smooth way.

Mikhail Pogorelyy

Methods for identification of condition-associated T-cell receptor clonotypes in RepSeq data

High throughput sequencing of antigen receptor repertoires (RepSeq) allows for the sequencing of millions of TCR/BCR sequences per sample. However, our ability to extract clinically relevant information from repertoire sequencing data is still limited. Here we present three computational approaches to identify vaccination, infection, cancer, or autoimmune disease-associated clonotypes from longitudinal RepSeq data (several timepoints after treatment for one donor), population RepSeq data (repertoires from patient cohort) and single repertoire samples.
First, we present a statistical model which detects clonal expansion by analysing TCR cDNA count data from RepSeq. We applied this model to TCRbeta repertoires of three twin pairs after yellow fever immunization (YFV17D). We identified 500-1500 expanded clonotypes in each donor, and validated them for YFV17D-specificity by three independent functional assays.
Second, we describe an algorithm to identify disease specific clonotypes using repertoires from cohorts of patients. A stochastic model of TCR recombination is used to identify clonotypes shared between larger numbers of patients than one could expect by chance. Using this model, we identified known public cytomegalovirus and ankylosing spondylitis-associated clonotypes in respective patient cohort repertoires.
Third, we extended this approach from the population level to single samples. We clustered TCRbeta clonotypes by significant sequence convergence, which is estimated and weighted from a stochastic TCR generation model. We show that identified clusters are abundant after YF-immunization and consist predominantly of YF-specific clones and almost absent before immunization.
Taken together, these approaches allow identification of disease-specific TCR variants using sequencing data only.

Michael Pun

Statistical Inference of B-cell Evolution during Affinity Maturation

Evolution of B-cells in the affinity maturation process plays a key role in the adaptive immune system response to infection in the human body. A quantitative understanding of affinity maturation would allow better understanding of B-cell life cycles, B-cell-pathogen coevolution, and could help guide vaccine development. One first step towards this understanding is a quantitative description of the phenotype under selection (i.e. binding energy) and how this phenotype relates to B-cell fitness. However, a description of affinity maturation is complicated by the high-dimensionality of genotype space, the complexity of B-cell structures, and the presentation of different epitopes to which the B-cell can bind. I am developing inverse statistical methods to investigate the phenotypic and antigenic forces that drive affinity maturation from data collected over a short time across multiple lineages undergoing contemporaneous evolution. I am testing the inference on synthetic data and plan later to test the inference on in vivo data sets such as the evolution of B-cell lineages in response to HIV vaccines.

George Sharonov

Intratumorally produced immunoglobulin repertoires

Although being in the front of immunology, in oncoimmunology B cells are much less studied compared to T cells, that have been in the focus of most immunotherapy studies resulting in most prominent clinical success. At the same time, it is clear that B-cells are also an integral part of antitumor immune response. B cells have multiple functions in immune-tumor interactions - including cognate antigen presentation, antibody and cytokines production, involvement in TLS functionality - that may either support tumor elimination or drive immune tolerance. This is manifested by strong predictive significance of certain immunoglobulin (IG) isotypes and B cell infiltrates. Recently developed bioinformatic tools allow extraction of intratumoral IG repertoires and isotypes from conventional RNA-Seq data. This opens up new opportunities for reviewing of available data and retrieving intratumoral IG repertoires with relation to the clinical outcomes. It has been done for analysis of melanoma samples from TCGA database. High intratumoral IG clonality has been found to be more significant positive prognostic factor than IG expression. High IgG1 clonality and expression level correlated and where associated with the best prognosis. On the opposite, IgA has low clonality and its expression is associated with negative prognosis in melanoma. Distinctive roles of B cells in some patients rise the possibility to stratify patients according to IG isotype prevalence and to boost antitumor immune response by either supporting or depleting B cells. Such prognostic values for IgG1 and IgA are also characteristic for some other cancer types, but are not universal. For example, both IgG and IgA may have positive prognosis for lung adenocarcinoma and negative for glioblastoma and kidney renal carcinoma. We will discuss how IG repertoire and isotypes may reflect B cell functions in antitumor immune response and potential use of this information for the therapy.

Vassili Soumelis

Systems approaches to dissect cell and molecular heterogeneity in the human tumor microenvironment

The tumor microenvironment can be seen as an organized structure containing a diver-sity of cells and cell types interacting with each other through communication signals. These forms so-called \cell communication networks", in which cells are the nodes and communication molecules are the edges. The complexity of those networks arises from the fact that 1) cells can harbor a multiplicity of states, and 2) edges are made of a large number of communication signals. We have built a systems biology framework to describe and analyze immune cell diversity and cell communication networks in human cancer. A rst aspect that we address is the diversity of immune cell types and cell states in the primary tumor microenvironment, by analyzing ex vivo human tumor samples using flow cytometry, in situ image analysis, population level and single cell RNAseq. We have analyzed the diversity of human dendritic cell subsets in breast cancer, and we could that they harbor dierent states in tumoral versus adjacent non-tumoral tissue. We are now dissecting dendritic cell diversity using single cell RNAseq. In parallel we address a second aspect, which is connecting dierent cells and cell types through ligand-receptor communication. We have developed an original methods to infer the connectivity of a given cell population with a large number of other cell types, based on transcriptomics proles. Such connectivity maps can be generated for any cell type, and can be applied to the tumor microenvironment to connect immune cells with each other and with tumor cells. Integrating that knowledge to reconstruct reliable cell communication networks characteristic of a given patient tumor could enable a detailed and personalized view of the tumor in order to guide therapeutic targeting.

Natanael Spisak

Evolutionary Paths in Affinity Maturation

The humoral response to an infection relies on the process of affinity maturation. In order to develop antibodies with good binding properties, B cells gather in germinal centers where they proliferate and acquire mutations. Good mutants are selected to further proliferate. This evolutionary process is fueled by somatic hypermutations (SHM) which occur at a very high frequency. We propose a repertoire-wide approach to study the properties of mutations on evolutionary trees. Our results help to understand the mutational landscape of the memory B cells and assert the relevance of a phylogenetic approach for identifying SHM hotspots.

Petr Sulc

Quantification of immunostimulatory patterns in RNA for innate immunity

Paul Thomas

TCR cross-reactivity and specificity in responses against tumors and pathogens

Nicolas VABRET

Viral mimicry by endogenous RNAs: an intracellular perspective

Pattern recognition receptors (PRRs) protect against host invasion by detecting specific molecular patterns found in pathogens and initiating an immune response. While microbial-derived PRR ligands have been extensively characterized, the contribution and relevance of endogenous ligands in PRRs activation during microbial infection remains largely overlooked. In this work, we characterize the landscape of endogenous ligands that engage RIG-I like receptors upon infection by a positive-stranded RNA virus, a negative-stranded RNA virus or a retrovirus. We found that several RNA polymerase 3 (POL3) transcribed RNAs, and in particular the family of small non-coding repeats Y-RNAs, bind and activate RIG-I upon infection. We further show that HIV-1 protein Vpr induces the degradation of RNA triphosphatase DUSP11, leading to an increase of Y-RNAs 5'-triphosphorylation that enables their immunogenicity. Importantly, altering DUSP11 expression is sufficient to induce a pro-inflammatory transcriptional program associated with HIV-1 infection and AIDS pathogenicity. Overall, our work uncovers the critical contribution of endogenous RNAs ligands to antiviral immunity and demonstrate the importance of this pathway in HIV infection. Further, the ability of endogenous triphosphorylated RNAs to activate innate receptors opens a potential role in priming the immune response in sterile conditions, as often found in tumor microenvironments.

Aleksandra Walczak

Identifying responding clonotypes in immune repertoires