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ABSTRACT BOOK

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METHODS, INSTRUMENTS AND RESULTS: THE RELEVANCE OF GOVERNING THE MACHINERY TO ADDRESS THE RIGHT QUESTIONS

M. Delledonne (Verona, IT)
University of Verona, Department of Biotechnology,
Strada Le Grazie 15, 37134, Verona

Since the completion of the human genome project in 2001, extraordinary progress has been made in genome sequencing technologies, which has brought the cost of sequencing a human genome down to around US$1,500, thus enabling the use of sequencing as a clinical tool[1].
Although exciting, these advancements are not without limitations. As new technologies emerge, existing problems are exacerbated or new problems arise. Current "Next Generation Sequencing" (NGS) platforms provide vast quantities of data, but the associated error rates (~0.1–15%) are higher and the read lengths generally shorter than those of traditional Sanger sequencing platforms, requiring careful examination of the results, particularly for variant discovery and clinical applications. Long-read sequencing technologies are becoming available, but their error rate and cost per gigabase are much higher, thus limiting their widespread adoption[2].
The fast and low-cost sequencing platforms now available are providing physicians with the tools needed to translate genomic information into clinically actionable results. However, there are many drawbacks in the current genome sequencing approaches that we must understand and, especially, keep into account when performing genome sequencing in the clinical settings. Understanding the current limitations of NGS and applying these technologies and their methods to tackle the appropriate question is therefore a fundamental aspect of precision medicine.

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GRAPEVINE BERRY TRANSCRIPTOMIC PHENOTYPIC PLASTICITY IN DIFFERENT ENVIRONMENTS ENLIGHTENS THE DIFFERENCES SENSED IN THE WINE

M. Pezzotti (Verona, IT)
S. Dal Santo[1], M. Sandri[2], S. Zenoni[1], G.B. Tornielli[1], P. Zuccolotto[2], M. Fasoli[1], G. De Lorenzis[3], L. Brancadoro[3], M. Pezzotti[1]

[1] Department of biotechnology, University of Verona, Italy
[2] Department Economy and Management, University of Brescia, Italy
[3] Department of Agricultural and Environmental Sciences Production, Landscape, Agroenergy, University of Milano, Italy

Grapevine, the most widely-cultivated perennial fruit crop, is also considered one of the most environmentally sensitive crop. It is characterized by remarkably phenotypic plasticity (i.e., the range of phenotypes a single genotype can express as a function of its environment) which in turn is believed to effectively buffer environmental extremes especially through transcriptomic and epigenomic reprogramming. Thus, the final phenotype (P) of a given grapevine plant is the result of the interaction between its genetic composition (G) and the environment (E).
We assessed the plasticity of the grapevine transcriptome during berry ripening in the red berry variety Corvina, cultivated in 11 different vineyards over a 3-year experimental plan (Dal Santo et al, 2013), here we analyzed Genotype x Environment (GxE) interactions in two grapevine varieties by characterizing their transcriptome plasticity when cultivated in different environments. Specifically, two genotypes (Sangiovese and Cabernet Sauvignon) were cultivated in three different locations in Italy (Bolgheri -littoral Tuscany-, Montalcino – Central Tuscany- and Romagna -foothill area-), trained in an almost identical manner, and sampled at four developmental stages over two grapevine growing seasons, 2011 and 2012, for a total of 144 samples that were analyzed by hybridization to a whole-genome microarray.
In order to study the relationships among differential gene expression profiles and environmental cues, we have developed a new statistical data mining tool based on data reduction approaches which allowed a dissection of the transcriptomic data into stage-specific, cultivar-related and GxE important clusters of gene expression.

Dal Santo S, Tornielli GB, Zenoni S, Fasoli M, Farina L, Anesi A, Guzzo F, Delledonne M, Pezzotti M **(2013) The plasticity of the grapevine berry transcriptome.** Genome Biol 14: r54

THE USE OF CIRCULATING TUMOR CELLS (CTCS) AS LIQUID BIOPSY TOOL IN CANCER RESEARCH AND CANCER DIAGNOSTICS

H. Wikman (Hamburg, DE)

University Medical Center Hamburg-Eppendorf
Institute of Tumor Biology
Martinistrasse 52
20246 Hamburg

Metastatic relapse is caused by single or clusters of disseminated or circulating tumor cells (DTCs and CTCs) that have escaped from the primary tumor and may spread to distant organs even before the first diagnosis of the disease. Thus DTCs and CTCs represent an intermediate step in the metastatic cascade and show characteristics found in established macrometastases. Following the first publication in 1981 (Dearnaley, et al., 1981) of the use of immunocytochemistry (ICC) for the detection of DTCs in the bone marrow (BM), numerous studies have been performed in various types of epithelial cancer, showing their clinical relevance. BM seems to be a common homing organ for DTC derived from various types of malignant epithelial tumors, including tumors which do not typically form metastases in bone like colon cancer. This suggests that BM might be the preferred reservoir for metastatic tumor cells from where they may re-circulate into other distant organs. DTC are present in BM samples of 15–40% of patients, even in patients with absence of clinical signs of distant metastases (stage M0) (Pantel and Brakenhoff, 2004). However, due to the invasive nature of BM aspiration, lately more studies have been performed on tumor cells found in the blood (CTCs).

The study of circulating tumor cells (CTCs) and circulating tumor-free DNA (ctDNA), known as liquid biopsy, has received increasing attention over the past years due to their many potential clinical applications in the individual management of cancer patients. Even though the CTC detection is currently more challenging than the isolation of ctDNA, from CTCs one can obtain more information at the DNA, RNA, and protein level and they can be used for functional analyses. In the metastatic setting, when no operation is anymore beneficial, if biopsies are taken, they are usually obtained from single sites. These biopsies are not always sufficient to recapitulate

the vast intratumoral heterogeneity, and therefore some druggable alterations might be missed. CTCs are released from all sites and representing the cells with a high metastatic potential and mirror the heterogeneity of the different tumor cells (Alix-Panabieres and Pantel, 2016).

In numerous studies both the number and the persistence of CTCs are associated with a worse prognosis. Especially in breast, prostate and colon cancer the presence of CTCs has been shown to be a strong and independent prognostic factor for both, early stage and metastatic patients (Pantel and Speicher, 2015). Furthermore, the possibility of repeated blood sampling allows disease monitoring in real time. Indeed, the enumeration of CTCs have shown to recapitulate the tumor response during treatment and that the elimination or decrease of CTCs following treatment is associated with improved clinical outcomes. Importantly, it has been shown that CTCs can show novel targetable alterations, such as HER2 overexpression, not seen in the primary tumor (Riethdorf, et al., 2010). Therefore, CTCs have been added to the TNM staging system in 2010 for breast cancer (cM0 (i+)). However, still little is known about the functional significance of CTC heterogeneity or phenotypic characteristics of CTCs, which establish metastasis (Pantel and Speicher, 2015).

In order to be able to detect one tumor cell per million hematopoetic cells highly sensitive assays are needed to study CTCs. Due to the rareness of these cells, usually a two-step approach is needed for the detection of the CTCs. After the first enrichment step the concentration of CTCs can be increased by several log units. Several specific enrichment methods are have been generated, including different types of density gradient separation, negative and positive cell depletion assays using e.g. immunomagnetic particles, filtration and magnetic affinity cell sorting (MACS) and fluorescence-activated cell sorting (FACS) based methods. The most commonly used approach to identify CTC is ICC with monoclonal antibodies against epithelial or tumor-associated antigens. Keratins are currently the most commonly used protein markers for the detection of CTCs and DTCs as keratins (K) are cytoskeleton proteins expressed in epithelial cells but usually absent in hemapoetic cells (Joosse, et al., 2015).

At present, the CellSearch system is the only FDA-cleared CTC detection method available on the market and is based on the isolation of EpCAM+/K+/CD45- cells. Interestingly, in NSCLC CellSearch generally detects less

CTCs compared to other tumour entities. Although infrequent, CTC detected by the CellSearch system has been significantly associated with shorter overall survival, indicating that also in NSCLC EpCAM+/K+ positive cells have prognostic value (Hanssen, et al., 2015). Similarly, we and others have shown that especially brain metastases patients often show less CTC when detected with classical epithelial markers, despite their usually extremely poor prognosis (Mego, et al., 2011). One explanation for these results could be that these cells have a heterogeneous expression of epithelial and mesenchymal markers indicating various levels of differentiation typical for cell that have undergone epithelial-to-mesenchymal transition (EMT). EMT has been linked with stem cell phenotype and more aggressive behaviour as well (Bednarz-Knoll, et al., 2012). Therefore, it would be important to learn more about the heterogeneous expression of CTC in order to use the CTC as an even more efficient aid in treatment decision and monitoring of the disease course. Furthermore, the usage of multiple cell surface proteins for CTC isolation and detection could increase sensitivity for CTC identification.

Both positive and negative interactions between immune and neoplastic cells play a major role during malignant progression and thus the immune response is considered one of the hallmarks of cancer. For the first time, a substantial number of patients with advanced NSCLC and melanoma have benefited greatly from immunotherapies, experiencing durable remissions and prolonged survival. Unfortunately, few robust markers exists that could predict, which patients will best respond to these therapies. Also here, the use of liquid biopsy might help in defining those patients profiting from this form of therapy.

In conclusion, CTCs represents an intermediate step in the metastatic cascade, and thereby can not only aid in defining the metastatic load of the patient, which might also be correlated to the peripheral immune surveillance, but can also support to determine the characteristics genomic profile found in established macrometastases and thus help the clinicians to define the optimal treatment plan for the patient.

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AGING, CLONAL HEMATOPOIESIS AND PRELEUKEMIA

L. Shlush (Rehovot, IL)

Weizmann Institute of Science
Department of Immunology
Rehovot, Israel

Acute myeloid leukemia (AML) has greater than 90% mortality among elderly (>65)1. Since de novo cases of AML present acutely it is impossible to undertake early diagnosis. AML is a clonal disease initiated by the accumulation of somatic mutations in preleukemic hematopoietic stem and progenitor cells (preL-HSPCs)2-4. However, somatic mutations can also be identified in large proportion of healthy individuals with clonal hematopoiesis of indeterminate potential (CHIP)5-8.

In an effort to distinguish the type of clonal hematopoiesis associated with natural human aging from that associated with progression to AML, we identified a unique cohort of 96 individuals from the European Prospective Investigation into Cancer and Nutrition (EPIC) study9 who enrolled as healthy individuals, donated blood, but then proceeded to develop AML on average ~6 years after enrollment (pre-AML cases). In parallel, enrollment blood samples of a set of 419 matched individuals who have not developed AML over an average of 11.6 years of follow-up (controls) were also analyzed. Deep error corrected sequencing (ECS) of 261 recurrently mutated genes in AML was used to study peripheral blood mutational profiles. In contrast to controls, pre-AML cases displayed a distinct age-correlated accumulation of somatic mutations and mutational signatures that resembled hematological malignancy datasets. Parameters such as clone size, variant allele frequency (VAF) distribution, specific mutations, and the number of mutations contributed to a highly predictive AML risk model. The model achieved sensitivity of 43.5%, specificity of 99%, positive predictive value (PPV) of 90.9%, negative predictive value (NPV) of 88.9%, and hazard ratio (HR) of 18.3 (95% confident interval 7.57-44. P<0.001).
The current model accurately predicted AML 5.6 years prior to diagnosis, thus providing a proof of concept that early prediction of AML development is feasible in high-risk cohorts.

LIFE IN MOTION
THE DEADLY JOURNEY OF METASTASIS

G. Scita (Milan, IT)

IFOM, the FIRC Institute of Molecular Oncology
& University of Milan, School of Medicine,
Department of Oncology and Hemato-Oncology-DIPO, Milan

Cells are dynamic entities, which constantly change to adapt to environmental conditions. A process that epitomizes such flexibility and plasticity is cell motility. The ability to acquire a coordinated mode of motion is paramount for proper tissues morphogenesis during development as well as for various processes in adult organism, including wound repair and the mounting of an immunological response. In pathology, the acquisition of motility is the most striking hallmark of advance tumours, which must spread and disseminate to distant site to form metastatic foci.

The molecular engine enabling the generation of forces that propel virtually any form of eukaryotic cell locomotion is the actin cytoskeleton. Actin is a small globular protein that upon binding to ATP can polymerize generating polar filaments, which display a remarkable structural and dynamic flexibility. The continuous polymerization and depolymerisation of polar actin filaments fuels a molecular tread milling process that is at the base of the generation of motion. The diverse structural architecture of actin filament is further exploited to generate protrusive forces in a variety of cellular processes and type of migration strategies.

How do cell move? The first migratory step of individual migratory cells is the extension of a polarized protrusion in the direction of motion. These protrusions can take different form and shape, but are invariable composed and propelled by dynamic actin filaments. Following protrusion formation, transient adhesion to substrates frequently mediated by specialized receptors of the integrin family ensues. These transient adhesion sites function as a mechanical clutch that enable to transform the pushing forces generated by the actin tread milling into net locomotion, de facto allowing a cells to move effectively. For the process to be completed, the

back of a cells must detach from the substrate through the action of elastic and contractile apparatus that literally acts as a spring to retract the rear and promote forward net motion. This canonical step-wise migration paradigm typically applies to cells crawling onto 2D substrates, such as fibroblasts. However, variations of this classical cycle of locomotion have clearly been observed, and are particularly relevant in driving the motility of poorly adherent cells, including the majority of our hematopoietic one and certain type of cancers. In addition, emerging evidence indicates that this relative simplified framework to describe how individual cell move represent only one of the many and alternative strategies adopted by cells and cell ensembles to migrate. Indeed, using intravitale microscopy and model systems that reconstituted motility in complex 3D environments have revealed how the modes of locomotion are extremely diverse and flexible. Cells in addition to move as single entities, may frequently migrate in collective fashion, forming group or chains linked by tight cell-cell interaction that in order to migrate must coordinate their individual motion with respect to their respective neighbours. Collective motility is, indeed, the primary mode of migration utilized by tumours of solid origin, which represent the vast majority of human cancer. Remarkably, however, tumours and normal cell alike are not constrained in their modes of motility. Rather they utilize a set of flexible or plastic migratory strategies, choosing the ones that better enable them to navigate through the complex and frequently changing tissue microenvironments. These adaptive responses have also important therapeutic consequences as they provide migratory 'escape' strategies after pharmaco-therapeutic intervention, by prompting alternative mechanisms of cancer cell dissemination in tissues that overcome single-pathway-hitting pharmacological weapons.

The main cause of mortality in cancer patients is the spreading of primary cancer cells that generate metastatic foci through a complex and still poorly understood process. One feature that contributes to make metastatic cell so deadly is their plasticity, i.e. the ability of cells to adapt to the microenvironment and change their identity to invade healthy tissues and proliferate. It is therefore essential to identify the various critical pathways and cellular processes that enable tumor cells to plastically adapt their motility modes during migration and invasion. Only after this knowledge has been gleaned, can pharmacological strategies targeted against multiple, deregulated tumor-specific migratory mechanisms, be devised and clinically tested with any hope of success.

We have recently shown, for example, that metastatic cancer cells adopt similar kinematic and dynamics behaviors of a crowd moving into confined spaces where the ability to flow and avoid jamming depends on their fluid mode of motion. Thanks to a set of integrated and multidisciplinary approaches at the crossroad of cell biology and soft matter physics, we provided evidence that the ability of group of cells to move collectively depends on their density and geometric shape. Through the employment of optical and electronic microscopy techniques, we found that a tissues composed on an epithelial cell ensemble typically undergoes a kinetic arrest, acquiring properties that are typically ascribed to crowded inert particles or viscoelastic materials. This liquid-to-solid or, as we called, unjamed-to-jammed transition has been proposed to ensure proper development of elasticity and of barrier properties in epithelial tissues, but also to act as formidable suppressive mechanism for the aberrant growth of oncogenic clones. Re-awakening of collective motility can occur through a reverse transition from a solid, rigid state to a fluid one, where large group of cells start flowing like current in the see, coordinating their locomotion over long range encompassing multiple cells. Such reverse Jamming-to-Unjamming Transition might also represent an alternative gateway to cell migration with respect to epithelial-to-mesenchymal transition (EMT) that enables cancerous tissues to escape the caging imposed by the crowded cellular landscape of mature epithelial. Indeed, if cells retain an epithelial phenotype throughout all stages of the metastatic process, then the idea of EMT as the sole or principal gateway to cellular migration does not pertain. Conversely, tumor cells may exploit this "mechanical flexibility" to execute key steps of the metastatic cascade without the need to change genetic make up and cell identity; hence requiring events that are significantly less drastic than EMT and the inverse MET to disseminate and to form metastatic foci.

HOW TO DEVELOP CLINICAL TRIALS IN THE NEW ERA OF MEDICINE

L. V. Marshall (London, UK)

The Royal Marsden
NHS Foundation Trust, London, UK

Cancer remains the leading cause of death in children beyond infancy in the developed world. This is despite intensive multi-modal therapy and steady improvements in survival outcomes over recent decades which has often resulted in the paediatric oncology paradigm held up as a worthy example of collaborative clinical trial efforts. There is thus still an urgent need to improve therapeutic options for children and adolescents with poor prognosis and high risk cancers. Stratified precision medicine approaches are revolutionizing treatment and outcomes in the adult cancer setting and it is crucial that this progress is harnessed for children. The advent of advanced molecular profiling has increased our understanding of actionable alterations in paediatric malignancies which may be targeted by novel agents developed for adults but with a mechanism of action relevant to paediatric tumour biology. Challenges include the relatively small individual patient populations in an already rare cancer setting, and diversity and heterogeneity of detected molecular alterations. There is thus also a need for more efficient drug development strategies to help progress promising drugs quickly and safely into combination approaches, avoiding treating children at sub-optimal doses in multiple unnecessary dose escalation cohorts, and better assessment of efficacy. In partnership with academia, pharmaceutical companies and regulatory agencies, specialist international paediatric oncology early phase trial consortia have, over recent years, been able to work towards refining the designs of early phase trials and overall development strategies. There has been a clear move towards running biomarker-rich, hypothesis-driven trials in relevant enriched population groups that move rapidly from dose-finding to expansion cohort phases and into combination settings, making the most of the data from every patient recruited, whilst (importantly) providing access to novel agents for more patients with an unmet medical need. Specific examples of such trials, and wider strategic initiatives, will be discussed.

HOW THE UK IS TACKLING THE CHALLENGE OF IMPLEMENTING PRECISION MEDICINE

S. Subramaniam (London, UK)

Cancer Research UK

Cancer Research UK is the largest fundraising medical research charity globally and second largest global funder of cancer research. These funds are used to drive research from basic to translational to clinical. The Experimental Cancer Medicine Centres (ECMCs) is a joint initiative between CRUK and Departments of Health for England, Scotland, Wale and Northern Ireland, funding centres of scientific and clinical excellence in translational research. This unique network and infrastructure provides a ready opportunity to introduce and embed precision medicine into the healthcare system. The UK operates on a National Health Service (NHS) funded solely by the Department of Health.

While the concept of precision medicine and targeted therapies is not new, a series of negative read-outs in targeted therapeutic trials in 2000-2010 demonstrated the importance of biomarkers and genomics to stratify patients. In 2010, CRUK's Molecular Pathology Survey identified that while molecular targeted therapies had been NICE-approved in solid tumours, there was no national programme of testing or commissioning available. This resulted in 2,500 patients a year potentially eligible for targeted therapies not receiving it due to lack of available molecular testing.

The first phase of CRUK's Stratified Medicine Programme (SMP1) was set up with the aim to demonstrate that national, high-volume molecular testing was feasible and to understand the requirements and challenges in a nationwide system. Using the ECMC network, SMP1 recruited patients with 6 tumour types from 8 clinical sites and excess biopsy material was sent to 1 of 3 NHS molecular genetics laboratories for molecular testing. SMP1 delivered on its aims consenting over 10,000 patients and testing over 9000 samples, demonstrating that with the appropriate infrastructure for both sample and data flow, molecular testing was possible on a national scale.

Following on from the success of SMP1, CRUK's Stratified Medicine Programme 2 (SMP2) is a multi-partner initiative designed to deliver high quality, cost-effective genetic testing for people with cancer across the UK. It screens non-small cell lung (NSCLC) cancer patients by next generation sequencing (NGS) for clinically-actionable gene changes in NSCLC that are likely drug targets. Based on these results, patients are recruited to a specific arm of the National Lung Matrix Trial (NLMT) linked to a targeted therapy, or to 'not actionable' (NA) arm (immunotherapy). The aim of SMP is to establish genomic screening at a national level within existing NHS pathways and ensure patients get access to novel targeted therapeutics as a result. This initiative is a highly effective partnership between the ECMCs, the NHS, pharmaceutical companies and the diagnostics industry. The SMP2/MATRIX framework and partnership model is based on SMP1 and the ECMC network, with patients recruited from over 50 hospitals across the UK and both clinical and genetic data are collected via a CRUK Data repository and embedded into the electronic patient records to inform clinical decisions. Since the start in 2015, the programme has evolved with increased understanding of the challenges faced at each stage of the pathway and through the introduction of incremental changes to improve the delivery of results in a clinically meaningful timeframe and increase translation onto NLMT. SMP2/NLMT is a collaborative and flexible model which is crucial for us to learn and adapt together with other UK and global precision medicine efforts.

TRANSLATIONAL SCIENCE IN THE ERA OF PRECISION MEDICINE

G. FitzGerald (Philadelphia, US)

Institute for Translational Medicine and Therapeutics,
University of Pennsylvania, Philadelphia, Pa., US

The number of new drug approvals has remained roughly constant over the past 65 years while the nominal cost of bringing a drug to market has escalated substantially. The likelihood of a drug that makes it into humans ultimately being approved by the FDA is ~10% with most of the failures occurring in phase 2. There has been a considerable erosion of the skill sets necessary to understand the mechanism of drug action in humans and to parse variability in drug response. We have focused on building such capacity in translational science with the foundation of the Institute for Translational Medicine and Therapeutics (ITMAT) 13 years ago. ITMAT prompted the launch of the Clinical and Translational Science Awards by the NIH and catalyzed translational science at Penn that delivered examples of precision medicine; for example, CAR- T cell therapy in leukemia and gene therapy for rare forms of blindness. Large randomized controlled trials deliver information on average signals of efficacy and safety, but despite their size, are liable to multiple defects in trial design that undermine even these conclusions. The challenge is to use translational science and diversified modelling approaches to develop algorithms predictive of drug response at the individual level. However, despite the scientific promise of precision medicine, the social challenge is to delivery affordable access to its discoveries. Drug pricing has become a major challenge and has evoked legislative, political and scientific responses. One aspect of the current model of drug development that merits reconsideration is our approach to intellectual property given the success of the altruistic sector in delivering drugs to market at a fraction of the advertised cost of the current model.

NEPHROLOGY: A GLANCE OVER THE FUTURE

G. Remuzzi (Bergamo, IT)

Department of Biomedical and Clinical Sciences - University of Milan
IRCCS Mario Negri institute for pharmacological research - Bergamo

The use of stem cells offers significant prospects for research and therapy. Currently, several cell types can be generated in vitro, due to methodological advances made in isolating and handling cells from various tissues and, even more importantly, because cell identity can now be changed by reprogramming and re-differentiating somatic cells. However, because organs and tissues are three-dimensional (3D), important aspects that influence organogenesis are missing in conventional two-dimensional (2D) cultures. The search for a solution to this problem has led to the development of 3D systems that enable the in vivo creation and growth of miniature organs called organoids, which are capable of developing organotypically and exerting organ-specific functions.

Organoids are usually generated from progenitor cells, which are either isolated from embryos or derived from pluripotent stem cells (PSCs). These methods evolved, methodologically and conceptually, from classical reaggregation experiments that demonstrated that dissociated cells from embryonic organs can reaggregate to re-form the original organ structure. Most organoid technologies are based on the use of exogenous growth factors that drive particular cell identities, as well as extracellular matrices, followed by reaggregation to stimulate cell movement and create self-organized 3D tissues.

As has been shown for other organs, evidence that kidney tissue may be capable of self-organization comes from early reaggregation experiments1, and recent studies have confirmed that kidney organoids can be obtained from various sources of progenitor or stem cells. Using a suspension of embryonic mouse cells, we recently generated kidney tissues that were able to filter blood in vivo, reabsorb macromolecules, and produce erythropoietin2. Building on this, we then constructed renal organoids using human amniotic fluid stem cells that vascularized upon transplantation, for-

med highly specialized human podocytes, and exerted nephron-specific functions3. Most importantly, the generation of kidney organoids, starting with human induced PSCs,4,5 has created significant opportunities for investigating organ development and tissue morphogenesis, modelling diseases, testing the efficacy and toxicity of drug compounds, and hopefully one day for creating tissues for tissue replacement therapy.
In this lecture, we will outline historical advances in the field and describe some of the major recent developments in 3D organoid formation, with a special emphasis on progress with generating kidney organoids. Finally, we underline current limitations and highlight examples of how organoid technology can be applied in biomedical research.

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CAR T CELL THERAPY BEYOND THE CD19 PARADIGM

M. Sadelain (New York, US)

Center for Cell Engineering
Memorial Sloan Kettering Cancer Center
New York, NY, US

Chimeric antigen receptors (CARs) are synthetic receptors that redirect and reprogram T cells to mediate tumor rejection. The most successful CARs used to date are those targeting CD19, which offer the prospect of complete remissions in patients with chemorefractory/relapsed B cell malignancies, especially acute lymphoblastic leukemia (ALL). The application of CAR therapy to solid tumor requires further evolution of CAR design and T cell engineering. To enhance the intrinsic (function, persistence) and extrinsic (action on the tumor microenvironment) potency of adoptively transferred T cells, we are investigating novel CAR designs and new approaches to genetically engineer CAR T cells. The constitutive expression of 4-1BBL increases T cell persistence and maintains function in T cells that express a CD28-based CAR. Furthermore, the constitutive display of 4-1BBL in CAR T cells trans-costimulates other tumor-infiltrating lymphocytes, diversigying and augmenting the anti-tumor response. T cells are typically engineered with -retroviral or other randomly integrating vectors (lentiviral vectors, transposons), which may result in variegated CAR expression and transcriptional silencing. Using CRISPR/Cas9, we have established conditions yielding efficient target gene disruption and CAR insertion at different loci. We have found that directing a CAR to the human T cell receptor (TCR) alpha chain (TRAC) locus not only results in efficient and uniform CAR expression in human peripheral blood T cells, but, remarkably, enhances T cell potency, vastly outperforming that of conventionally generated CAR T cells. Placing CAR expression under the control of the TCR alpha promoter minimizes tonic signaling and allows effective CAR internalization and re-expression following antigen engagement, which is associated with diminished T cell exhaustion. These findings illustrate the potential of genome editing to advance immunotherapies.

HOW COLONIZATION BY MICROBIOTA IN EARLY LIFE SHAPES THE IMMUNE SYSTEM

R. S. Blumberg (Boston, US)

Division Chief, Gastroenterology, Hepatology & Endoscopy
Brigham and Women's Hospital
Professor of Medicine, Harvard Medical School, Boston, US

Humans are colonized by trillions of microorganisms that include fungi, archaea, viruses, protozoans and bacteria. The highest number of these microbes can be found in the intestinal tract and in the colon in particular. The past decades have shown a growing interest in studying the phenotype and function of the microbiota in the gut and its roles in immunity and health outcomes. Analyses of microbiota composition in adult humans have revealed numerous associations between specific bacterial phyla and disease and elucidated a number of pathways by which this may occur. In the gut itself, microbial dysbiosis is strongly associated with inflammatory bowel disease development. This latter example has been quite instructive as it been difficult to determine whether the microbial alterations are primary and causative or secondary events that are derived from intestinal inflammation itself.

Of recent interest is the possibility that many of the disease associated microbial changes that have been observed and their immune consequences may originally develop during the earliest days of life. During the early post-natal period of life, the microbiota is in the process of colonizing the host and its composition is highly unstable. This time of early life bacterial colonization also correlates with the development of the immune system and its education to tolerate its environment to fight pathogens and avoid allergy and autoimmunity. Increasing evidence shows that certain immune cell populations can be regulated by microbiota in a time restricted fashion. Further, perturbations of the microbiota during a specific early life time-period can have persisting effects on the immune system later in life. This has predicted the existence of a window of opportunity during the early life of the host which is amenable to environmental manipulation.

Early post-natal life is a key time for the development of the immune system and colonization of the host by microbiota.
Recent studies have shown that specific limbs of the immune system can be regulated by microbiota in a time restricted period during this period. Studies in mouse models have shown that perturbations of the microbiota during early life can cause immune effects that can persist into adulthood and create increased host susceptibility to certain diseases as recently shown by us for CD1d-restricted invariant natural killer T cells. iNKT cells have been linked to numerous diseases including inflammatory bowel disease, asthma, contact dermatitis and eosinophilic esophagitis.
Understanding the early life events associated with iNKT cell behavior therefore has important implications for the prevention and treatment of numerous diseases.

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