

# Immunological Genome Project and systems immunology

Tal Shay<sup>1</sup> and Joonsoo Kang<sup>2</sup>

<sup>1</sup> Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA

<sup>2</sup> Department of Pathology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, USA

**Immunological studies of single proteins in a single cell type have been complemented in recent years by larger studies, enabled by emerging high-throughput technologies. This trend has recently been exemplified by the discovery of gene networks controlling regulatory and effector  $\alpha\beta$  T cell subset development and human hematopoiesis. The Immunological Genome Project (ImmGen) aims to decipher the gene networks underpinning mouse hematopoiesis. The first phase, completed in 2012, profiled the transcriptome of 249 immune cell types. We discuss the utilities of the datasets in high-resolution mapping of the hematopoietic system. The immune transcriptome compendium has revealed unsuspected cell lineage relations and the network reconstruction has identified novel regulatory factors of hematopoiesis.**

## **A transcriptome compendium of mouse hematopoiesis**

Classic immunology studies with a laser focus on a particular protein or biological process are becoming increasingly complemented by systems immunology studies that provide robust insights to understand fully the inner workings of the immune system. With technological advances, a systems immunology approach is feasible for individual laboratories, and not just for large consortia. However, the scope of individual enterprise still remains mostly restricted to a particular cell lineage [1–3], and as a consequence, variations on shared modular gene networks embedded in multiple cell components of a system are largely inaccessible. These variations are often the main drivers of diversity in cell types and the basis for division of labor within a cell system. As examples, cell type-specific function of SMAD3 downstream of transforming growth factor (TGF) $\beta$  is dictated by lineage-specific master transcription factors (TFs) [4]; and context-dependent functions of interferon-regulatory factor (IRF)4 can be accounted for by its association with distinct activator protein (AP)-1 factors [2,5]. In practice, the ability to survey widely and yet recognize patterns of biological significance as they emerge necessitates a collective, in which uniform data generation and quality control go hand-in-hand with input from immunologists and computational biologists who can process the data and articulate clear paths towards deducing biological meanings of the emerging patterns [6]. There are several initiatives that

have tried to achieve this goal. For example, RefDIC, an open resource compendium of quantitative mRNA and protein profile data of immune cells [7], and ImmGen, which is arguably the most comprehensive effort to date, and is the focus of this review.

ImmGen is a consortium whose primary objective is to establish a comprehensive, public compendium of gene networks operating in the mouse hematopoietic system – the components, their properties, and their behaviors upon perturbations [8]. The consortium is made up of immunologists with expertise in specific immune cell subsets working with computational biologists in pursuit of network models to determine gene circuit architectures underpinning complex biological systems. Data so far have been restricted to RNA because it is the only reproducibly quantifiable trait of cell subsets present at limiting numbers in a system-wide scale. Affymetrix microarrays were chosen as the data collection platform [8]. Alternative assays, such as RNA-seq, have been used to verify the data quality (<http://www.immgen.org/Protocols>), and upon optimization are likely to be more prominently utilized in the future. The ImmGen site ([www.immgen.org](http://www.immgen.org)) is a portal with several data browsers, each allowing the user to study a unique aspect of the ImmGen dataset (Table 1, Box 1). There were 78 038 visits to the ImmGen site from 98 countries during 2012.

Although the overarching thrust of ImmGen has been to map at high resolution the global interconnectivity of gene networks across cell constituents, solutions to investigator-driven, cell type-specific queries have fortified the network tree with rich details. Here, we highlight newly emerging themes in the gene circuitry driving the hematopoietic system, constructed from the ImmGen mRNA profiles data amassed over the past 6 years; in large part using the network modeling program termed Ontogenet specifically developed for ImmGen (Figure 1A). We first describe Ontogenet and the regulatory program it predicts, and then present examples for each of the three major thematic studies of ImmGen to date. We also discuss the integration of ImmGen with other datasets.

## **Ontogenet – a novel method for reconstructing regulatory networks in tree-structured datasets**

Ontogenet is a new method that combines linear regression with the tree structure of the dataset to predict a set of transcriptional regulators that would best account for the expression of each module [9]. A module is a set of genes that are coexpressed across the dataset. Regulators are

Corresponding author: Kang, J. ([Joonsoo.Kang@umassmed.edu](mailto:Joonsoo.Kang@umassmed.edu)).

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**Table 1. Data browsers of the ImmGen dataset, available at [www.ImmGen.org](http://www.ImmGen.org).**

Browser name	Purpose
Gene skyline	Display expression profile of a single gene in a group of cell types
Gene families	Display interactive heatmaps of predefined gene families
Gene expression map	Color-coded expression of genes along the genome
Population comparison	Find differentially expressed genes between user defined groups of cells
Modules and regulators	Browsing modules of coexpressed genes, their annotation and predicted regulators
RNA-seq	Visualize RNA-seq data of B and CD4 <sup>+</sup> T cells
Human mouse comparison	Display expression profile of a single gene in seven cell types measured in humans and mice; the expression matrix of the module to which this gene was assigned in the specified species; and the expression of the corresponding orthologous genes in the other species.

selected from a predefined list of factors that regulate gene transcription (TFs and chromatin modifiers). Ontogenet is specifically devised to address some of the challenges – and leverage some of the unique power – of studying transcriptional programs in cell lineages. First, Ontogenet can identify a whole set of ‘equivalent’ regulators, whereas other approaches (somewhat arbitrarily) choose only one representative. This is more consistent with the dense interconnected nature of regulatory circuits that control cell states. Second, Ontogenet allows us to choose a regulator in a context-specific manner, assuming that it may be relevant to the regulation of a gene module only in some cell types, but not others. Third, Ontogenet uses the lineage tree to guide its search for a regulatory program, by preferring (but not mandating) programs where ‘close’ cells in the lineage share regulatory mechanisms.

### ImmGen regulatory model

The transcriptional response of the mouse hematopoietic system was separated into modules of coexpressed genes at two levels of resolution. At the lower resolution, 81 coarse-grained modules, including genes with broadly similar expression patterns, were defined. Each coarse-grained module was further separated into fine-grained modules, representing smaller groups of genes with more coherent and tighter expression patterns, resulting in 334 fine-grained modules. For example, the coarse-grained module C33 is induced in B cells, and separated into fine-grained modules that are induced in different subsets of B cells, or at different progenitors of B cells (see [Figure 1B](#) in [Box 1](#)). Ontogenet reconstructed a regulatory model to each of these modules. The regulatory models included 554 of the 580 candidate regulators; at least 175 (32%) of which have not been previously implicated in the hematopoietic context they are predicted to regulate. Some known regulators have not been identified by Ontogenet; in most cases because they are expressed at low levels (biologically

or due to suboptimal probe sets design) and have been filtered out.

### Gene network architecture properties responsible for diversity of hematopoietic cell types and their function

One of the major goals of the ImmGen Phase 1 has been to define and sort operationally discrete cell subsets within a defined functional lineage as a systemic baseline measurement of transcriptome complexity. Compound perturbations of the system followed by iterative transcriptome samplings (Phase 2) will in principle yield the complete dynamic range of the system transcriptome and all dominant regulators. This task is to a large degree constrained by the availability of reagents (antibodies to cell surface markers or engineered cell type-specific reporters) to segregate live cell subsets from the whole population, as well as by the size of the cell subset.

So far, most ImmGen data are generated from adult (4–6 weeks old) male C57BL/6 mice. At the last release of the first phase of ImmGen (June 2012), the dataset comprises 816 arrays of 249 cell types, sorted from 27 tissues in 11 laboratories. All cell types were harvested under standard procedures, and the array hybridization was done in a central facility, in order to ensure minimal experimental biases. The lineage relations between the cell types are described in [Figure 1B](#). The lymphoid branch of the lineage tree is much more structured and includes more known progenitors, whereas the myeloid branch has few known progenitors, and many cell types with no known relations between them. Thus, currently, the lymphoid system is comparatively information-rich for studying transcriptional evolution along differentiation, whereas the myeloid lineage is ripe for identifying novel precursor-progeny and inter-subset lineage and functional relations.

The Phase 1 ImmGen studies can be broadly divided into three categories of survey: (i) gene expression profiles across hematopoietic subsets [10–14]; (ii) developmental intermediates [12,13,15,16]; and (iii) subset-specific perturbations [10,15,17]. Here, we highlight the major themes that have emerged in each category and readers are directed to the published reports for more details.

#### *Transcriptome-based lineage relations in the hematopoietic system*

**Myeloid versus lymphoid transcriptional program.** When the two major branches of the hematopoietic system, lymphoid and myeloid, are compared, gene modules and regulators most tightly linked to each can be discerned ([Figure 2](#)). Many of the pan-myeloid regulators with strongest activity are known regulators, such as *Batf3*, *Cebpa*, *Cebpb*, *Mafk*, and *Relb* [18]. The shared modules reflect not only the properties of the pioneering progenitors of the lineage but also common functions. For example, module C24 that is induced in all myeloid cells is predicted to be activated in all myeloid cells by *Ctbp2*, *Creg1*, *Tcfe3*, *Cebpa*, *Xbp1*, and *Bach1*. C24 contains complement components, genes with antiviral activity, and tissue remodeling genes.

The global analysis also reveals known regulators, previously implicated to have only a limited sphere of influence over the lineage, which may have broader activity. An

## Review

example is Rbpj, a factor necessary for induction of Notch target genes, and previously shown to control CD11b<sup>+</sup> dendritic cells (DCs) differentiation and function [19], but identified by Ontogenet as an activator of both CD11b<sup>+</sup> and CD11b<sup>-</sup> DCs in the plasmacytoid DC-specific module F150. Pan-lymphoid modules are fewer (only three coarse-grained modules classified as lymphoid specific, compared to 12 classified as myeloid specific), in agreement with the high degree of interlymphoid lineage divergence, compared to divergence in the myeloid lineage [9]. Ets1 is the only well-characterized regulator of lymphocytes identified in this manner.

**Myeloid lineage subsets distinction and regulation.** In the myeloid lineage, although subsets within the macrophage or DC sublineage show extensive transcriptome variability underpinning subset diversity at a resting state, the number of genes uniquely expressed in either sublineage is constrained, with the expression of 14 and 24 genes restricted to macrophages and classical DCs, respectively [11,16]. This is very different from the inter-sublineage differences

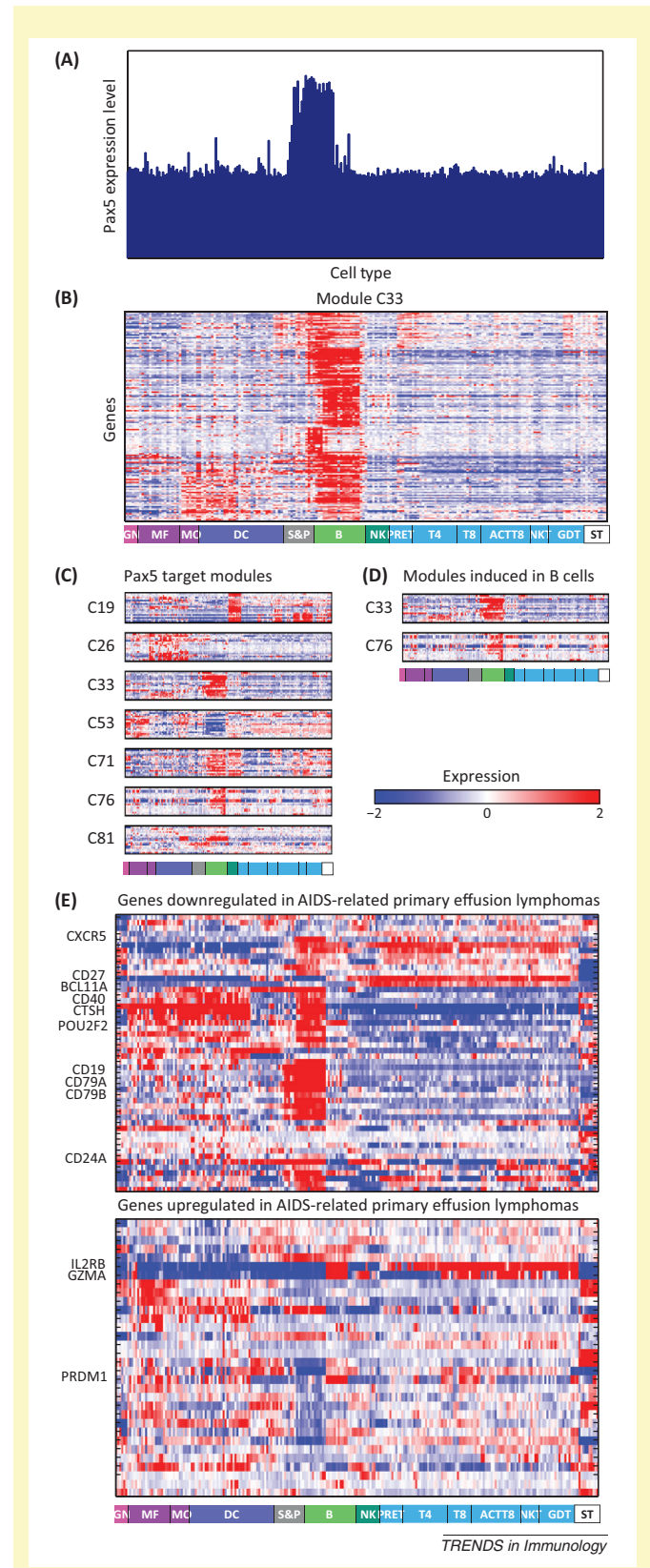
in the lymphoid lineage that can involve thousands of genes (e.g., B vs T [20],  $\gamma\delta$  T vs  $\alpha\beta$  T [12]). Core gene signatures unique to myeloid sublineages identify new markers (MerTK and CD64 for macrophages; cKit and BTLA for DCs) that should aid in more pure separation of

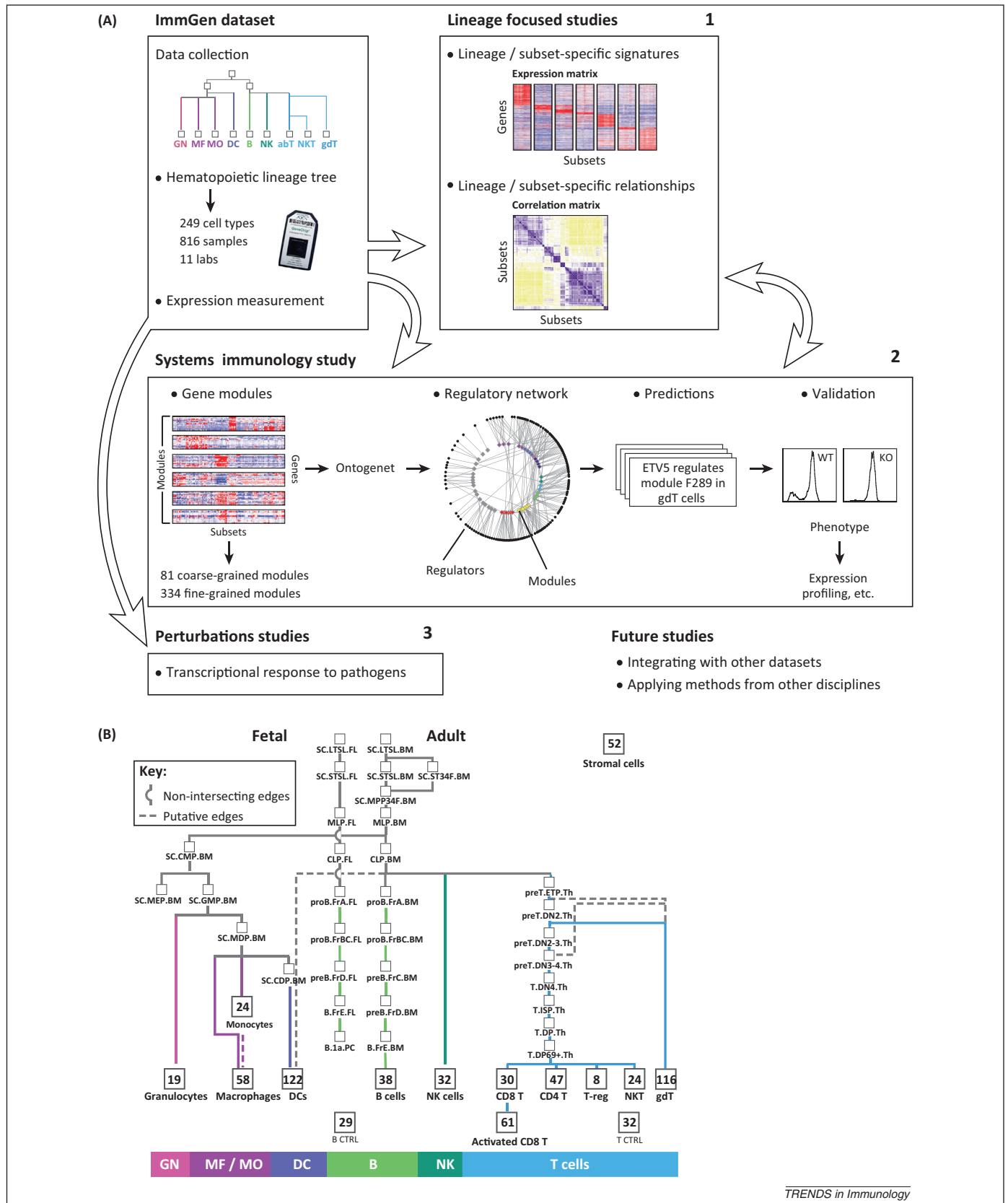
### Box 1. Usages of ImmGen

We envision several applications of ImmGen by the community, most of which are easily accomplished by one of the ImmGen browsers (<http://www.immgen.org/>):

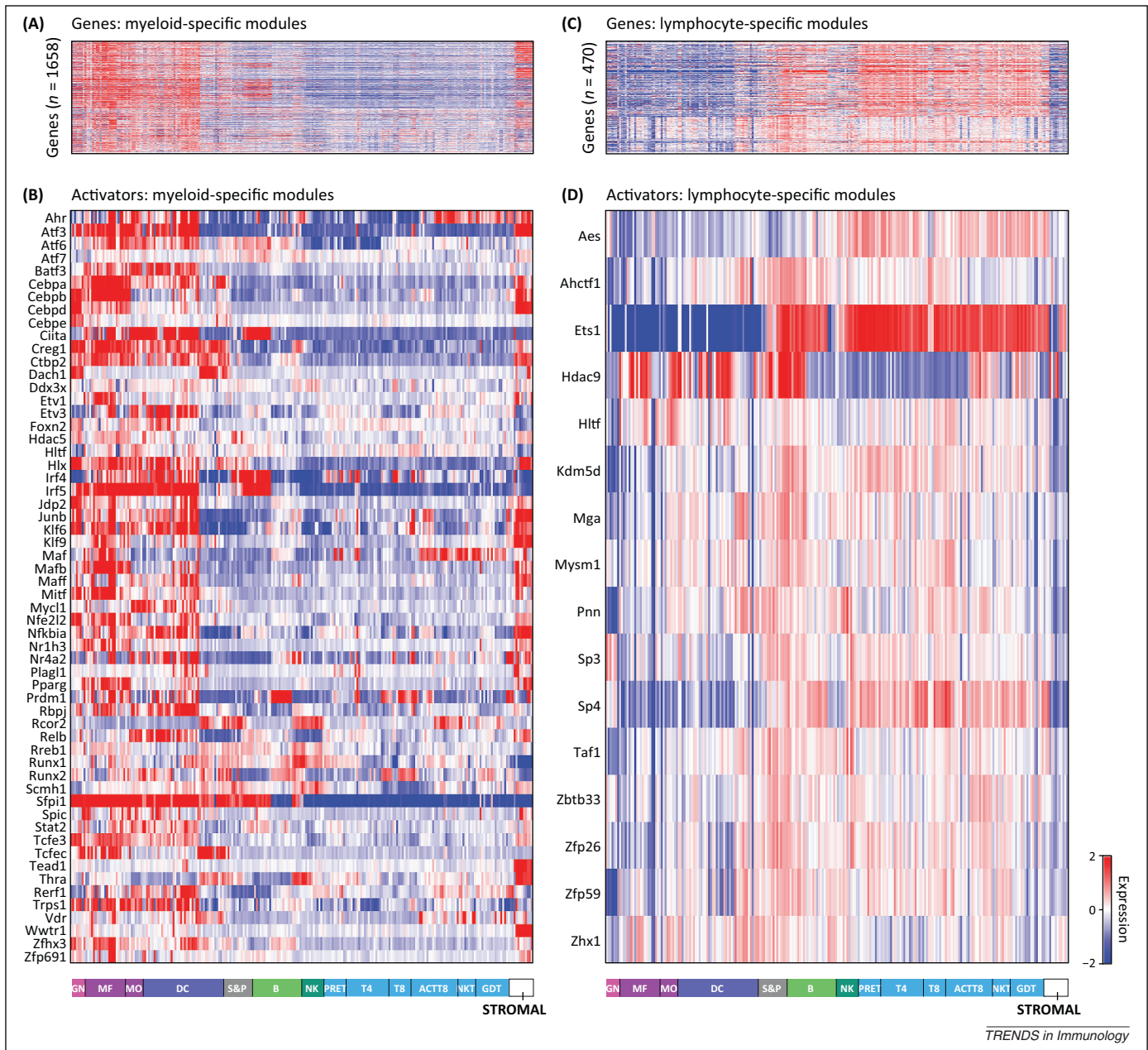
- (i) Determining immune cell types in which a gene of interest is expressed, using the Skyline viewer (e.g., Pax5, Figure 1A).
- (ii) Identifying the module to which a gene of interest is assigned, that is, which genes are coexpressed with a gene of interest, using the Modules viewer (e.g., C33, the Pax5 module, presented as a heatmap, Figure 1B). This will also give information about the predicted regulators for the module of this gene, by Ontogenet, enriched sequence motifs, and binding events.
- (iii) Tracking the modules predicted to be controlled by a regulator of interest (e.g., Pax5, Figure 1C).
- (iv) Identifying differentially expressed genes between groups of immune cell types, using the Population Comparison browser.
- (v) Browsing modules induced in specific cell types (e.g., B cells, Figure 1D).
- (vi) Extracting the expression patterns of a group of genes of interest across all ImmGen cell types. This is possible for several predefined groups of genes using the Gene families browser. Although this application is not currently available for user defined groups, it can be performed using custom-made scripts [e.g., genes downregulated (top) or upregulated (bottom) in AIDS-related primary effusion lymphoma samples compared to other tumor subtypes and normal B lymphocytes [44], Figure 1E].
- (vii) Determining conservation of expression pattern of a specific gene or module in the human immune system using the human mouse comparison browser.

**Figure 1.** Examples of ImmGen application for gene-focused studies. (A) Bar chart of Pax5 expression in immune cells, using the Skyline viewer. (B) A heatmap of the gene expression of the genes in coarse-grained module C33. Each row is a gene and each column is a cell type. Color bar below represents cell lineage. (C) Heatmap of coarse-grained modules predicted to be controlled by Pax5. Each row is a gene and each column is a cell type. Color bar below represents cell lineage. (D) Modules that are induced in B cells. Each row is a gene and each column is a cell type. Color bar below represents cell lineage. (E) Heatmap of genes downregulated (top) or upregulated (bottom) in AIDS-related primary effusion lymphoma samples compared to other tumor subtypes and normal B lymphocytes [44]. Only genes assigned to ImmGen modules are shown, sorted by module number. Selected gene symbols are shown to the left.





**Figure 1.** The ImmGen compendium and its analysis. **(A)** ImmGen dataset (top left), and the three types of studies performed by the ImmGen consortium: (1) Lineage focused studies that have identified lineage-specific genes (e.g., heatmaps of gene expression across cell subsets), have described the relation between lineages (represented by Pearson correlation matrix) and assigned cell types to the lineages tree. (2) System studies that have defined modules of coexpressed genes across the entire dataset and reconstructed the regulatory program of the modules using Ontogenet. The resulting regulatory network has described organizational principles governing the differentiation within the immune system, and has identified candidate novel regulatory factors that can be experimentally verified. (3) Perturbations studies that have investigated the transcriptional response to pathogens. ImmGen dataset can be integrated with other datasets, and methods from other disciplines can be applied to it, to gain new biological perspectives (future studies). **(B)** The hematopoietic lineage tree constructed by ImmGen. Big rectangles with numbers represent a group of cell types, with the number of replicates listed. Small rectangles represent single cell type, typically with three replicates. Lines/edges between rectangles represent differentiation steps.



**Figure 2.** Lymphoid- and myeloid-specific modules and activators. Conserved pan-regulators of lymphoid and myeloid branches can be gleaned from the comparison of lymphoid- and myeloid-specific modules. **(A)** Expression matrix of the genes in the modules that are induced in myeloid cells (Coarse modules 24, 25, 28, 29, 30, 31, 32, 45, 49, 74, 75, and 77). **(B)** Expression matrix of the activators assigned to the modules that are induced in myeloid cells with a maximal activity weight  $>0.05$  (arbitrary threshold). **(C)** Expression matrix of the genes in the modules that are induced in lymphoid cells (Coarse modules 16, 21, and 22). **(D)** Expression matrix of the activators assigned to the modules that are induced in lymphoid cells with a maximal activity weight  $>0.5$ . Blue-red color bar shows relative expression level.

the cell subsets from other myeloid cells [11,16]. Any one macrophage subset is significantly more divergent in a tissue-restricted manner to other macrophages types than inter-DC cell subset variations determined in the same way [11]. Moreover, all migratory DCs in tissue-draining lymph nodes share common gene signatures, accented by genes that may impose immune tolerance to self [16]. These results reinforce a pattern of strong influence of tissue environments and cell trafficking in specifying myeloid immune surveillance function, with most likely parallel environmental adaptations by lymphoid cell subsets, as illustrated by distinct gene expression profiles of T cell subsets in the gut in comparison to the pattern

associated with corresponding populations in other anatomical locales [12,13,21].

Gata6 and Spi-C are examples of novel regulators of macrophages as identified by Ontogenet. Although Gata6 is a regulator of macrophage-specific modules, it may be particularly important for peritoneal macrophages based on the expression pattern. Similarly, Spi-C may perform specialized function for splenic red pulp macrophages [11]. Fine modules characteristic of distinct DC subsets have common predicted regulators. Most of these regulators are known, such as Batf3, Irf8, and Ciita, but many more have not been characterized yet in the DC context, suggesting that the subset-specific regulators in concert with the

pan-DC lineage factors induce transcriptional programs that define functionally distinct cell types [16].

**Lymphoid lineage subsets distinction.** Within the thymus, several subsets of T cells arise with diverse effector or regulatory functions [12,22,23]. Historically, T cells have been segregated by the type of T cell receptor (TCR) expressed ( $\gamma\delta$  or  $\alpha\beta$  TCR) or by function (helper/regulatory or cytotoxic). Module analysis reveals a different property for lineage separations. Overall, thymocyte subsets can be clustered based on innate (functionally active within hours or a couple of days post-infection or environmental alterations, with preprogrammed immune effector repertoires, and no significant difference between initial and subsequent responses) or adaptive (builds up to maximal responses within several days with acquired effector functions driven by pathogen elicited milieu, and subsequent response faster and stronger than initial response) classifications rather than the type of TCR expressed. For example,  $\gamma\delta$  T cells expressing V $\delta$ 6.3 TCR are intrathymically programmed to secrete both interleukin (IL)-4 and interferon (IFN) $\gamma$  by inducing Zinc finger and BTB domain-containing protein 16 (Zbtb16) (Promyelocytic leukemia zinc finger, PLZF), a TF exclusively expressed in  $\alpha\beta$  T cells with innate-like function [23]. The prototype of PLZF<sup>+</sup>  $\alpha\beta$  TCR<sup>+</sup> cell types is the invariant  $\alpha\beta$  natural killer (iNKT) cells expressing the canonical V $\alpha$ 14 TCR. V $\delta$ 6.3<sup>+</sup> and V $\alpha$ 14<sup>+</sup> T cell subsets have a significant overlap in transcriptomes [12,13], likely accounting for their common functional capabilities and potential functional redundancies [24]. The transcriptional program induced in  $\alpha\beta$  iNKT cells is made up of elements of innate NK cells and adaptive  $\alpha\beta$  T cells [13]. Together, these results raise the possibility that the primary transcriptome-based subset stratification in lymphocytes is dictated by distinct molecular programs associated with innate versus adaptive immune function.

#### *Gene network architectures during hematopoietic cell differentiation*

Gene network modeling is capable of identifying novel candidate regulators for modules induced in specific cell types, as well as modules and regulators repeatedly used during development and in fully differentiated cell states. An intriguing example for cell type-specific module and its novel candidate regulators is module C60, induced only in fetal liver stem and progenitor cells, but not in adult bone marrow stem and progenitor cells. C60 contains members of the H19 network (Igf2, H19, and Dlk1) implicated in the control of fetal and postnatal growth in mice, and Plag1, a known regulator of the H19 network [25]. Ontogenet predicts Plag1 and Hmga2, a target of Lin28b [26], to be regulators of C60. Lin28b has been shown to be a determining factor of fetal lymphopoiesis [27] and induces the expression of genes, such as Zbtb16, necessary for the generation of innate lymphocytes, which appear to originate preferentially during fetal gestations or early postnatally [27–29].

As a prototype of repeatedly utilized regulators, TCF-1 (transcription factor 7, T cell specific; encoded by *Tcf7*), a T cell lineage specification factor [30,31], possesses far-ranging activities in diverse cell types (e.g., intrathymic  $\gamma\delta$

effector cell subset differentiation [32], control of NK cell receptor expression [33], maintenance of CD4<sup>+</sup> T helper (Th)17 cells [34], and generation of CD8<sup>+</sup> memory  $\alpha\beta$  T cells [35]). Tcf7 is identified as a regulator in two coarse-grained modules and 18 fine-grained modules. TCF-1 controls diverse biological processes by extensive interactions with, and modulations by, cofactors that themselves are often cell type-restricted (e.g., Sox13 in  $\gamma\delta$  T cells). Predictably, many of the regulators that specify cell lineage fate, such as Irf4, forkhead box (Fox)p3, and Ror $\gamma$ t, are characterized by complex interactomes as the driving force of their finely tuned functional activities [2,5,36].

The detailed cellular characterization of developmental intermediates in lymphoid cell sublineages offers an ideal system to probe the dynamic molecular basis of cell lineage specification. As a framework, gene transcription dynamics associated with hematopoietic cell differentiation are generally modeled on three sequential processes: (i) a rapid and transient onset of activities of transcription factors responsible for turning on genes associated with differentiated states in immature lineage precursors; (ii) suppression of genes associated with the immediate precursor state or alternate cell fate choice; and (iii) long-lasting induction of genes associated with differentiated states [12,15,37–40]. In the thymus, early thymic progenitors generate innate  $\gamma\delta$  T and adaptive  $\alpha\beta$  T cells. The general model applies well to the innate T cell development where the lineage specific TFs (e.g., Sox13, Etv5, Zbtb16, and Tox2) are turned on very early in differentiation and are shut off upon maturation into specialized effectors in the thymus [12]. A similar force acts on innate-like  $\alpha\beta$  iNKT differentiation where key early regulators (e.g., Zbtb16) are downmodulated upon maturation [13]. By contrast, in adaptive T cell differentiation the apex regulators do not achieve maximal induction until well after T cell lineage commitment and their expression is maintained in mature T cell subsets that exit the thymus. However, many of these regulators are turned off after T cell activation and differentiation into specialized effector subsets [15]. Hence, both innate and adaptive T cells follow the general dynamics of TF controlled lineage differentiation; the major difference being that for innate T cells, the differentiation window is compressed and mostly restricted to the thymus, whereas for adaptive T cells, their differentiation is marked by more than one wave of core regulator expression, and is not fully terminated until the memory phase. In this sense, the transcriptome dynamics in development fully accounts for the fast and slow nature of innate and adaptive lymphocytes, and provide the molecular blueprint for the widely held view that the thymus exports memory-like innate T cell subsets.

#### *Temporal evolution of lymphoid regulatory modules post pathogen encounters*

Initial infection studies involving innate (NK), innate-like (NKT), and adaptive (CD4<sup>+</sup> and CD8<sup>+</sup>  $\alpha\beta$  T cells) lymphocytes have provided preliminary leads to the molecular rewiring involved in the generation of short-lived effectors and long-lasting memory cells of the lymphoid lineage [10,13,17].

A hallmark of the adaptive T cell response is the massive expansion of pathogen-specific T cells within days of

infection. Predictably, antigen-specific CD8<sup>+</sup> T cells responding to *Listeria monocytogenes* infection maximally induce transcription of a large cluster of genes within 48 h, with the majority involved in proliferation, activation (TCR and cytokine mediated), and increased metabolism. The memory phase (>45 days post-infection) involves reversing much of the initial superinduction and expression of a limited number (13) of memory-specific genes, including Bcl2 and Cdh1 [17]. Ontogenet analysis identified known players in effector and memory CD8<sup>+</sup> T cell subset differentiation (e.g., Tbx21, Erg2, Egr3, Prdm1, and Tcf7), as well as candidate regulators of short-term effector/memory cells, such as Rora, Tox, and Zeb2. Interestingly, CD8<sup>+</sup> T cell gene clusters associated with the memory phase were operational in a subset of innate-like NKT cells and activated  $\gamma\delta$  T cells [17], suggesting that similar functions in different cell types are programmed by common regulators (e.g., fine-grained module F99). Conversely, innate NK cells (independent of virus encounters) and intestinal intraepithelial  $\gamma\delta$  T cells are most similar to effector CD8<sup>+</sup> T cells. Thus, the preprogrammed innate lymphoid subsets as a group possess a full spectrum of adaptive effector and memory CD8<sup>+</sup> regulatory programs, reinforcing the theme that cell function is a central determinant of the transcriptome, and by extension, cell lineage relatedness. Note however, that how each cell type attains particular functional specialization can be unique. This array of unique responses likely minimizes the impact of variants that can escape canonical immune responses.

### Insights from integrating ImmGen with other datasets

The power of ImmGen dataset is not only in the analysis of the data within it, as described above, but in the integration with external systemic datasets, which can amplify informational outputs and yield new paradigms. An example is the comparison of ImmGen with a similar, although much more limited, human dataset termed differentiation map or 'D-MAP', collected an expression compendium of 39 cell types (211 samples) from human immune and hematopoietic lineages [40]. Comparison of ImmGen and D-MAP expression profiles show that most orthologous genes between humans and mice show similar expression patterns in the immune lineages, although there are some differences [41]. The recently published ENCODE data [42], which aim to map the functional elements in the human genome, is another prominent candidate for integration.

A recent study, termed differentiation map or 'D-MAP', collected an expression compendium of 39 cell types (211 samples) from human immune and hematopoietic lineages [40]. Comparison of ImmGen and D-MAP expression profiles show that most orthologous genes between humans and mice show similar expression patterns in the immune lineages, although there are some differences [42].

### Concluding remarks

The scope, uniform data collection and quantitation procedures, and centralized regulatory model construction of the ImmGen compendium have established the baseline measurement of variations in the hematopoietic transcriptomes that allow for many novel analyses. There

remain some gaps in the survey (e.g., fetal hematopoietic system, nonlymphoid tissue-resident hematopoietic cell types). In many ways, the completion of Phase I is the starting point for unraveling the molecular circuits dictating the versatility and fitness of mammalian immunity. This effort will necessitate incorporating new methods from other fields (Box 2), and combining transcriptome dynamics with global chromatin analyses (epigenetic and core TF occupancy) across resting and perturbed hematopoietic cell subsets. The resultant context-dependent inducible gene modules will provide testable leads to lineage-specific mechanistic studies of cell development and function, as well as novel, network-integrated drug targets for hematopoietic diseases. Given some notable shortcomings of mice as a model for human immunology and disease processes [43], a community effort to build a human ImmGen seems warranted to exploit fully the information explosion that is expected to follow during the next phases of ImmGen.

### Box 2. Applying methods from other disciplines to the ImmGen dataset

The scope and structure of the ImmGen dataset invite application of computational methods not generally familiar to most immunologists. One of the enriching aspects of the consortium is that dialogues between groups of different scientific backgrounds can produce new questions and applications of methods not strictly related to gene expression analysis to provide possible answers. For example, can the information content of the transcriptome of each cell type in the system approximate its level of differentiation, more specifically the pluripotency of hematopoietic stem cells (HSCs) versus the restricted lineage potency of its progenies? Hematopoiesis is a process in which an HSC has all the information required to generate blood and immune cell types, whereas terminally differentiated cells can only create the same cell type. HSCs and terminally differentiated cells carry the same genetic information, but their transcriptome (and other parameters) is different, and likely responsible for the difference in the lineage potential. Information Theory is a mathematical field that studies the information content of sets of numbers [45], which can be applied to address the basis for the hierarchical nature of hematopoiesis.

As a second example, all modeling methods of transcriptional regulators assume that the transcriptome of a cell, which includes tens of thousands of genes, is a function of the activity of a limited set of regulators; typically dozens to hundreds of genes. The Junta problem is defined as finding a function whose output is dependent on only a constant number of input parameters; typically much smaller than the number of output parameters [46]. This is an open problem with a lot of research in Machine Learning. Defining a regulatory model is equivalent to finding a function whose output is the transcriptome of a cell, and input is the expression of the regulators, making it a Junta problem. The ImmGen dataset suggests a case study to apply heuristics developed for the Junta problem on a real life problem.

In the last example, cell types of the immune system are mostly defined by a somewhat arbitrarily selected set of the cell surface proteins they express. It was shown that there are intermediate cell types between well-characterized cell types and lineages [47]. This can be interpreted as a result of the arbitrary definition, or of incompletely characterized cells. However, the cells may follow the chemical reactivity–selectivity principle [48], stating that the more reactive the molecule, the less selective the response. A system optimized for speed must trade off on the selectivity, typically by producing intermediate outputs, or have an output production pathway that is not specific. The functional cell should be able to generate a myriad of outputs to potentially variable inputs (e.g., pathogens). Hence, applications of this principle to the ImmGen dataset can produce testable hypotheses.

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## References

- Lee, Y. *et al.* (2012) Induction and molecular signature of pathogenic TH17 cells. *Nat. Immunol.* 13, 991–999
- Ciofani, M. *et al.* (2012) A validated regulatory network for Th17 cell specification. *Cell* 151, 289–303
- Amit, I. *et al.* (2009) Unbiased reconstruction of a mammalian transcriptional network mediating pathogen responses. *Science* 326, 257–263
- Mullen and Alan, C. *et al.* (2011) Master transcription factors determine cell-type-specific responses to TGF- $\beta$  signaling. *Cell* 147, 565–576
- Glasmacher, E. *et al.* (2012) A genomic regulatory element that directs assembly and function of immune-specific AP-1–IRF complexes. *Science* 338, 975–980
- Benoist, C. *et al.* (2012) Consortium biology in immunology: the perspective from the Immunological Genome Project. *Nat. Rev. Immunol.* 12, 734–740
- Hijikata, A. *et al.* (2007) Construction of an open-access database that integrates cross-reference information from the transcriptome and proteome of immune cells. *Bioinformatics* 23, 2934–2941
- Heng, T.S.P. *et al.* (2008) The Immunological Genome Project: networks of gene expression in immune cells. *Nat. Immunol.* 9, 1091–1094
- Jovic, V. *et al.* (2013) Identification of transcriptional regulators in the mouse immune system. *Nat. Immunol.* <http://dx.doi.org/10.1038/ni.2587>
- Bezman, N.A. *et al.* (2012) Molecular definition of the identity and activation of natural killer cells. *Nat. Immunol.* 13, 1000–1009
- Gautier, E.L. *et al.* (2012) Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat. Immunol.* 13, 1118–1128
- Narayan, K. *et al.* (2012) Intrathymic programming of effector fates in three molecularly distinct [gamma][delta] T cell subtypes. *Nat. Immunol.* 13, 511–518
- Cohen, N.R. *et al.* (2012) Shared and distinct transcriptional programs underlie the hybrid nature of iNKT cells. *Nat. Immunol.* 14, 90–99
- Malhotra, D. *et al.* (2012) Transcriptional profiling of stroma from inflamed and resting lymph nodes defines immunological hallmarks. *Nat. Immunol.* 13, 499–510
- Mingueneau, M. *et al.* (2013) The transcriptional landscape of  $\alpha\beta$ -T cell differentiation. *Nat. Immunol.* 14, 619–632
- Miller, J.C. *et al.* (2012) Deciphering the transcriptional network of the dendritic cell lineage. *Nat. Immunol.* 13, 888–899
- Best, J.A. *et al.* (2013) Transcriptional insights into the CD8<sup>+</sup> T cell response to infection and memory T cell formation. *Nat. Immunol.* 14, 404–412
- Satpathy, A.T. *et al.* (2012) Re(de)fining the dendritic cell lineage. *Nat. Immunol.* 13, 1145–1154
- Lewis and Kanako, L. *et al.* (2011) Notch2 receptor signaling controls functional differentiation of dendritic cells in the spleen and intestine. *Immunity* 35, 780–791
- Painter, M.W. *et al.* (2011) Transcriptomes of the B and T lineages compared by multiplatform microarray profiling. *J. Immunol.* 186, 3047–3057
- Shires, J. *et al.* (2001) Biological insights into TCR $\gamma\delta$ <sup>+</sup> and TCR $\alpha\beta$ <sup>+</sup> intraepithelial lymphocytes provided by serial analysis of gene expression (SAGE). *Immunity* 15, 419–434
- Rothenberg, E.V. *et al.* (2008) Launching the T-cell-lineage developmental programme. *Nat. Rev. Immunol.* 8, 9–21
- Godfrey, D.I. *et al.* (2010) Raising the NKT cell family. *Nat. Immunol.* 11, 197–206
- Pereira, P. and Boucontet, L. (2012) Innate NKT $\gamma\delta$  and NKT $\alpha\beta$  cells exert similar functions and compete for a thymic niche. *Eur. J. Immunol.* 42, 1272–1281
- Varrault, A. *et al.* (2006) *Zac1* regulates an imprinted gene network critically involved in the control of embryonic growth. *Dev. Cell* 11, 711–722
- Kiel, M.J. *et al.* (2005) Spatial differences in hematopoiesis but not in stem cells indicate a lack of regional patterning in definitive hematopoietic stem cells. *Dev. Biol.* 283, 29–39
- Yuan, J. *et al.* (2012) *Lin28b* reprograms adult bone marrow hematopoietic progenitors to mediate fetal-like lymphopoiesis. *Science* 335, 1195–1200
- Grigoriadou, K. *et al.* (2003) Most IL-4-producing  $\gamma\delta$  thymocytes of adult mice originate from fetal precursors. *J. Immunol.* 171, 2413–2420
- Haas and Jan, D. *et al.* (2012) Development of interleukin-17-producing  $\gamma\delta$  T cells is restricted to a functional embryonic wave. *Immunity* 37, 48–59
- Weber, B.N. *et al.* (2011) A critical role for TCF-1 in T-lineage specification and differentiation. *Nature* 476, 63–68
- Germar, K. *et al.* (2011) T-cell factor 1 is a gatekeeper for T-cell specification in response to Notch signaling. *Proc. Natl. Acad. Sci. U.S.A.* 108, 20060–20065
- Malhotra, N. *et al.* (2003) A network of High Mobility Group box transcription factors programs innate IL-17 production. *Immunity* 38, 681–693 <http://dx.doi.org/10.1016/j.immuni.2013.01.010> Epub 2013 Apr 4
- Held, W. *et al.* (1999) Clonal acquisition of the Ly49A NK cell receptor is dependent on the trans-acting factor TCF-1. *Immunity* 11, 433–442
- Muranski, P. *et al.* (2011) Th17 cells are long lived and retain a stem cell-like molecular signature. *Immunity* 35, 972–985
- Jeannot, G. *et al.* (2010) Essential role of the Wnt pathway effector Tcf-1 for the establishment of functional CD8 T cell memory. *Proc. Natl. Acad. Sci. U.S.A.* 107, 9777–9782
- Fu, W. *et al.* (2012) A multiply redundant genetic switch 'locks in' the transcriptional signature of regulatory T cells. *Nat. Immunol.* 13, 972–980
- Miyamoto, T. *et al.* (2002) Myeloid or lymphoid promiscuity as a critical step in hematopoietic lineage commitment. *Dev. Cell* 3, 137–147
- The FANTOM Consortium and Riken Omics Science Center (2009) The transcriptional network that controls growth arrest and differentiation in a human myeloid leukemia cell line. *Nat. Genet.* 41, 553–562
- Amit, I. *et al.* (2007) A module of negative feedback regulators defines growth factor signaling. *Nat. Genet.* 39, 503–512
- Novershtern, N. *et al.* (2011) Densely interconnected transcriptional circuits control cell states in human hematopoiesis. *Cell* 144, 296–309
- Shay, T. *et al.* (2013) Conservation and divergence in the transcriptional programs of the human and mouse immune systems. *Proc. Natl. Acad. Sci. U.S.A.* 110, 2946–2951
- The ENCODE Project Consortium (2012) An integrated encyclopedia of DNA elements in the human genome. *Nature* 489, 57–74
- Davis, M.M. (2008) A prescription for human immunology. *Immunity* 29, 835–838
- Klein, U. *et al.* (2003) Gene expression profile analysis of AIDS-related primary effusion lymphoma (PEL) suggests a plasmablastic derivation and identifies PEL-specific transcripts. *Blood* 101, 4115–4121
- Shannon, C.E. and Weaver, W. (1949) *The Mathematical Theory of Communication*, University of Illinois Press
- Mossel, E. and Servedio, R.A. (2003) Learning juntas. In *Proceedings of the 35th Annual ACM Symposium on the Theory of Computing*, pp. 206–212
- Bendall, S.C. *et al.* (2011) Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. *Science* 332, 687–696
- Mayr, H. and Ofial, A.R. (2006) The reactivity–selectivity principle: an imperishable myth in organic chemistry. *Angew. Chem. Int. Ed. Engl.* 45, 1844–1854