Canadian Human Immunophenotyping Symposium

September 26, 2015
Montréal, Québec, Canada

Meeting summary and future perspectives

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A. Executive Summary

The Canadian Human Immunophenotyping Symposium was a one-day workshop held at the McGill University Health Centre on Sept. 26, 2015. The goal of the event was to assess the need for and community's interest in a uniform approach to human immunophenotyping in Canada with respect to development of guidelines, best practices and/or SOPs for acquisition, analysis and reporting of human immunophenotyong data; as well as to explore the establishment of a national committee and/or centre to promote the harmonization of human immunophenotyping across Canada with links to international efforts. The approximately 60 invitees included a cross-section of Canadian academic researchers engaged in clinical and basic human immunophenotyping research, flow cytometry centre managers, representatives from relevant industries, as well as key stakeholders, including the Canadian Institutes of Health Research (CIHR), the Canadian Society for immunology (CSI) and the Federation of Clinical Immunological Societies (FOCIS). The event included a plenary session with keynote speakers actively involved in human immunophenotyping consortia from the US (Philip McCoy, HIPC) and Europe (Ola Winqvist, ENTIRE), four breakout sessions and a plenary consensus building session. This report provides a summary of each session and the overall recommendations emerging from the event.

Overall outcome:

There was an overwhelming consensus among the group that there is a strong need for harmonization and development of uniform approaches and protocols for flow cytometry. The benefits of a centralized repository of SOPS, operational and analytical standards as well as recommended antibody panels for core applications were clearly voiced by the group. It was considered more realistic to initially focus on harmonization and developing guidelines and best practices, rather than formal standardization and certification. There was general agreement that we should do an environmental scan and consolidate information about best practices, SOPs and panels to find common ground among the different groups/silos already conducting such work, independent of platforms or application. There was also consensus that standards need to be developed for data analysis, including development of automated gating procedures and best practices for reporting, annotating and storing data. Longer term, there is interest in centralized data repositories and potentially certified centers. There was strong support for the formation of a national committee, The Canadian Human Immunophenotyping Consortium (CHIC), with a mandate to develop a human immunophenotyping research resource, to develop and maintain a set of guidelines, best practices and SOPs for conducting and reporting human

immunology research. The group gave the organizing committee a mandate to form an interim working group to begin this process.

Recommendations/ action items:

1. Form an interim working group to start the Canadian Human Immunophenotyping Consortium (CHIC). This interim working group is now active and includes the Symposium organizing committee along with a few additions to balance data analysis and clinical cohort expertise, as well as appropriate regional representation (CHIC committee: Amit Bar-Or, Ryan Brinkman, Deborah Burshtyn, Cynthia Guidos, Tania Watts + 2 TBA).
2. Identify funds to hire a project manager.
3. Conduct an environmental scan and consult stakeholders.
4. Develop a working plan and form subcommittees to collect, evaluate, develop and/or disseminate harmonized best practices/guidelines. Once established, CHIC would serve, via an interactive website, as a resourced for these harmonized protocols and SOPs.
5. In the medium term, develop a funding plan and resources for data deposition/centre development and to develop training and educational activities.
6. In the long term, consider developing and certifying core immunophenotyping centers and training programs.

B. Historical background

In November 2014, the CIHR Institute for Infection and Immunity (III) hosted a Human Immunology Workshop in Toronto. The purpose of this Workshop was to reunite researchers and clinicians from the human immunology community, as well as government-related officials, to identify the major research directions for the greater community in the area of human immunology research. While a number of important topics were discussed, the issue of standardization and harmonization in human immunophenotyping was identified as an important strategic area of future funding and research activities.

Following this Workshop, a proposal emerged for holding of the first Canadian Human Immunophenotyping Symposium, which was ultimately held on Saturday, September 26, 2015, at the newly inaugurated Centre for Translational Biology, Research Institute of the McGill University Health Centre (Montreal, Canada). Guided by the experience of US and European representatives, as well as major industrial partners, the overarching goal of this Symposium was to bring together the major stakeholders in human immunology from across Canada to discuss, develop and

implement standards or “best practices” for immunophenotyping in human immunology and clinical research. This Symposium would also explore the possibility of establishing a national reference consortium to promote these guidelines.

C. Statement of Symposium objectives

The objectives of the Symposium were:

1. To discuss the development of recommended standards (best practices) for immunophenotyping cores/platforms in Canada
2. To discuss developing standards/guidelines for big data storage, retrieval and analysis
3. To discuss the establishment of a national reference centre/national committee (one or more) which would promote guidelines (best practices) for all those working in the area of human research/clinical immunology.
4. To provide a framework to draft a document with recommendations for platforms in immunophenotyping coming out of symposium

To help achieve these objectives, the Symposium was structured in a manner to facilitate discussions and establishment of clear goals and action items. To this end, a number of interactive, thematic discussion groups enabled participants to interact and identify specific future steps. Moreover, the Symposium had the pleasure to welcome two keynote speakers, namely Dr. Phil McCoy (NIH, Human Immunophenoptyping Consortium, HIPC), and Dr. Ola Winqvist (Karolinska Institute, ENTIRE consortium), who updated us on the US and European Human Immunophenotyping initiatives, respectively.

D. Summary of Keynote Speaker Presentations

Dr.Marc Ouellette, Scientific Director of the CIHR Institute for Infection and Immunity (III)

- Dr. Ouellette discussed the funds that are available for various initiatives of the Institutes. For 2016/2017 there is about $4M available and III is participating in initiatives in Microbiome/gene interactions (1 Million commitment) and in chronic inflammation. For 2018/2019 there will be cross-cutting multi-institute

initiatives totaling $18-19 million, and III will compete to bring their initiatives into some of the cross-cutting themes.

- CIHR is embracing the data revolution along with NSERC, Health Canada, SHRC and CFI. Genome Canada and CIHR are collaborating on a National strategy for bioinformatics and Computational biology. Dr. Ouellette also mentioned the Canadian Research Data Centre network.

Dr. Winqvuist, ENTIRE consortium, Sweden

- Dr. Winquist described the experience of organizing the ENTIRE network in Europe. He also pointed out the huge gap between academic immunology and clinical practice. He suggested that there were lessons to be learned from clinical evidence-based medicine as espoused by Cochrane and Jorgen Nordenstorm.
- Dr. Winquist suggested that we might take the same approach to deciding on standards for human immunology, including determining the quality of evidence available to date, i.e. A systematic review: 1. Formulate the question; 2. Comprehensive search, 3. Unbiased selection and abstract process; 4. Critical appraisal of the data; 5. Synthesis of the data, 6. Interpretation of results.

Key features of ENTIRE study:

Technical parameters:

- Uses whole blood assays
- Uses FITMAN panels (see Fitman report attached) with some change in clones;
- Choice of clones/fluorophores important: Eg. old CCR7 didn't work; new CCR7 PE 150503 exhibited huge difference with much improved sensitivity for donor to donor variation
-The panel has been established at 8 participating centres.
- SOP constantly updated at www.entire.net.edu

Study plan and status:

- ENTIRE started with a multicenter study of 24 healthy donors at each of 8 sites. To date the experience is that there is a huge spread of T cell subset frequencies across healthy donors.
- Goal is to define reference values based on more than 350 healthy individuals, and move to disease cohorts once parameters for healthy donors are established.

- Working with healthy children has been a challenge
- Data analysis templates are a challenge
- Data sharing: need approval of health authorities

Lessons from ENTIRE

- Grass-roots commitment of team members and highly cooperative spirit enable considerable progress with very limited resources
- Dr. Winquist pointed out that there is a large difference between Standardization and Harmonization. Standardization is extremely difficult. ISO certification is unrealistic for flow, while harmonization of SOPs is possible
- Systematic discussion and agreement upon definition of biomarkers is reasonable, but identical instrumentation and reagents would be unrealistic and there is a lack of calibrators for standardized assays. For harmonization of Immune monitoring see: Science TM 9 Nov 2011 Vol 3 issue 108 p 44

Strengths of ENTIRE:

FOCIS Centres of Excellence
Not publication-driven
Quarterly meetings
Yearly school
Short term scientific missions
Lack of funding- people not in it for the money
Focus on training and education:
    Senior researchers- master classes
    Junior fellows- short stay visit to learn
    Workshops for 200 people

Weaknesses:

Adding in new centers after the initial process began was thought to be disruptive
Lack of funding
Dedicated Mab provider
Time optimism (i.e.; things took longer than anticipated).

Dr. Philip McCoy, NIH, Human Immunophenotyping Consortium (HIPC)

Critical issues:
Multiple variables were identified that can contribute to variability; Identifying and finding solutions for these sources of variation is critical. Participants must follow the SOP, starting with the blood draw right down to which needle size and which tube, approach to cell preparation, defining panels, flow cytometer choice and set-up.

Key variables include: History of person; Blood draw technique (IV or butterfly differ; anti-coagulant use); sample preparation (fresh vs. cryopreserved; Ficoll vs. RBC lysis); choice of panels/reagents (evaluate fluorchromes and clones); implementation of assay (staining and acquisition); choice of flow cytometer (standardization of instrumentation; thresholding or number of events); approach to analysis.

Models identified - Centralized or Remote:
1. Central reference facility: more regimented, uniform; need to establish central lab and shipping of specimens; may need to work with stored rather than fresh samples
2. Remote sites: can run fresh samples; anticipate greater technical variation
*3. Hybrid Model: may be reasonable compromise; take advantage of implementing uniform r*eagents, consensus SOPs, references standards. All go to 2 Central facilities for validation, then redistribution of reagents and SOPs to remote sites where SOPs will be followed for on site analysis. Data can also be sent back for centralized analysis.

Even implementation of consistent 'Good practices' across laboratories doesn't guarantee consistent results across labs. Nonetheless, there needs to be a minimum of 'Good practices' in each participating lab. Then step-up to harmonization across groups;
Then eventually advance to standardization.

FITMAN- HIPC panel was agreed to: the original core of 8 colors was deemed acceptable (even if not 'best'). Flexibility to add beyond original 8 parameters/colors, as long as these not impacted.

Additional observations:
For clinical studies, fresh blood generally preferred. For clinical research, often use cryopreserved samples. Enables side by side analysis including serial and replicate samples (to limit inter- and intra-assay variability). However cell losses expected (e.g.

through ficoll); can't do neutrophils; certain markers altered by processing (e.g.; CD62L disappears on frozen samples).

Common problems with multisite trials: Variations in shipping times/exposures; Choice of using stabilized whole Blood or PBMC; if cryopreserved cells strict SOP for thawing required as well. Transfix (stabilized blood) or lyophilized cells (Beckman coulter product). Avoid different people doing the gating (subjective manual gating). Careful naming of protocol. It is critical to adhere to the SOP.

Choice of clones can make a big difference (e.g. CD38, HB7 and HIT2 from BD), hence importance of being consistent. Generally opt with clones providing better separation, though consider compromise if single acceptable clone easier to use throughout.

Lyoplate study: takes advantage of lyophilized reagents in plates. If chosen, need to pick one for whole study, avoid switch between suppliers.

US clinical laboratories have checklists for compliance etc. and get proficiency tested and need for accreditation. Good to have enforcement.

Aspects of instrument standardization: Not practical to dictate one identical instrument in multiple academic settings. Brand is less important than the configuration. Wavelength and power of lasers can influence performance of some antibodies. Elements to consider as part of chosen acquisition strategies: threshold settings (important to set voltages for FSC/SSC); use of live gating; local versus central gating (central gating results in smaller CVs); defining appropriate number of events (total or a specified population; gated or ungated); use of time as a parameter to exclude fluctuations; use of absolute counts or percentages; doublet discrimination critical; thoughtful approach to order of running tubes.

FLowCAP: Bioinformaticians who are developing automated gating for flow. This is improving over time. Within a couple of years automated gating will be the way to go, but people must follow the SOPs. Ryan Brinkman (Vancouver) is one of the leaders of this group.

Follow-up note: 6 Swedish centres have the HIPC panel in clinical use and the European group has now collected 300 healthy donors Immunophenotypes that they will be publishing shortly (reported by Thomas Giese on FCE committee phone call).

Take home messages combined from both Winquist and McCoy Talks:

- Harmonization/Standardization is a Continuum; Tests in clinical practice: require standardization; in clinical research: more of harmonization; in basic academic research: individually developed SOPs.
- Keep Management group of the Network small; 8-10 people
- Frequent face-to-face meetings (with good wine and food!)
- Short term scientific missions
- Its an iterative process
- SOP compliance is a major issue
- Include foot soldiers as well as generals in the planning
- Instrumentation: Difficult to dictate use of a specific brand and model Configs, filters, lasers, and laser power need to be similar, or preferably identical; Rare or difficult to gate populations remain problematic; Consider automated gating.

E. Thematic breakout session summaries

Theme 1: Definition of best practices in multi-parametric flow cytometry in health and disease - *facilitated by Ciro Piccirillo*
Theme 2: Creation of a national repository of validated panels and standard operating procedures (SOP) - *facilitated by Amit Bar-Or*
Theme 3: "Big" data repository and management – *facilitated by Debby Burshtyn*
Theme 4: Establishment of a national standards consortium - *facilitated by Tania Watts*

Each group was asked to 1. Define the issues, 2. Create a working vision of what a consortium could achieve and 3. Identify action items if possible. The summaries of the themes revealed significant overlap particularly for Themes 1 and 2 but are covered here separately to underscore the parallels and various points raised in each group.

E.1 Theme 1 *Definition of best practices in multi-parametric flow cytometry.*

E.1.1. Background

There is a range of techniques that can be used for multi-parametric immunophenotyping and while many of these are used for various experimental and clinical purposes, flow cytometry is currently the most prevalently used technology. Theme 1 focused on the definition of the best practices for multi-parametric flow

cytometry. The group attempted to define the current needs in this area, the various factors that challenge the community, and the possible need/mandate of an eventual national reference centre/consortium. The breakout session focused on the general guidelines that should govern multi-parametric flow cytometry in any given platform or national standardization initiative. Attendees at this breakout session originated from academia (basic and clinical research) and industry. Some short and long term goals as well as recommendations for future actions were also discussed. A summary of the deliberations is found below.

E.1.2. The needs of the community and the mission and priorities of the Consortium

The group unanimously voiced a crying need for harmonized and uniform approaches and protocols for flow cytometry. The group manifested a strong desire for formal, established links between existing centres performing similar studies. Investigators often feel that they are working in a vacuum with regards to standardization practices in flow cytometry, and few strategies exist to harmonize practices across many labs. The need for a centralized repository of SOPS, operational and analytical standards as well as multi-antibody panels (cocktails) was clearly voiced by the group, and would likely impact the work of investigators across many different biomedical disciplines. A centralized “know how and how to” repository would significantly facilitate research activities across various labs in the human immunology community, particularly as these relate to data integration/analysis in multi-centric studies.

At the moment, there is no centralized database of standard operating procedures (SOPs), other than the one’s found in local research labs/units, if any at all. Investigators often comply with protocols that “work”, although these are likely not standardized across multiple labs. The group reiterated the importance of not “reinventing the wheel” as several other initiatives and consortia around the world have already established robust and applicable guidelines in multiple areas of investigation (i.e. Euroflow, ENTIRE, FOCIS/HIPC, neoplasm initiative, McGill ETP, CTRNP).

The overarching objective of the current initiative would be to define best practices in flow cytometry and establish specific standard practices for pre-analytical variables [sample identification/procurement, tissue type, cell isolation, processing and storage, fresh vis-à-vis frozen cells, labeling etc.), instrumentation calibration, antigen identification, multi-color panel design, panel validation process, reagent handling, data acquisition and analysis (gating strategies), data reporting, and training. The long-term objective is to create a national reference centre (i.e. multi-centered platform) as a resource for all those in human immunology research

(fundamental or clinical). The goal is to become a resource for human immunology researchers (training). How these guidelines could be applied in research and clinical labs remains to be seen. The need for formal training programs (workshops, webinars, courses etc.) is also implored.

E.1.3. The challenges and obstacles

The group had a long discussion on the numerous challenges and obstacles that investigators face. Notwithstanding the need for harmonization and standardization, there is a perceived difficulty to establish such best practices across Canada considering the diversity of cell types, tissue types and disease processes examined (e.g.; standards for pathologists). The group asked whether different sets of standards will be needed depending on cells, tissues and disease studied (each expert have their sophisticated panel). While cell-specific and disease-specific variables clearly come into play, some over-arching variables impact results in flow cytometry.

E.1.4. Variables identified that could be addressed by harmonization

1.4.1 Instrument variability

- Considered to be a major problem if you want to standardize
- How to deal with travelling specimens examined on different instruments
- Should we plan for core labs? Same expert people and same machines
- Standardization in shipping, thawing, freezing
- Need for intra and inter-instrument configuration
- Creation of a calibrating reference point

1.4.2 Design and use of multi-marker panel

- Nature of surface/intracellular marker: level of expression and quality of Abs
- Definition of fluorochrome panels: how colors fit in simple vs. complex panels?
- Standardization of basic panels depending on the protocol/application used
- General guidelines for instrument configuration and calibration
- Designing protocols/panels that are flexible: such as the 8-color standard FitMan panel, with room to add markers for individual studies
- In a clinical setting, some tests are not paid for and limited by cost.
- Marker reproducibility between companies: identity of clone and fluorochromes
- Labeling approaches: surface, intracellular, and intranuclear. Uniform protocols.

1.4.3 Sample-related variables

- Different sample types: whole blood, PBMC, fractionated cells, tissue biopsy
- Pre-processing issues: method of sample procurement influences downstream analyses (for example, using butterfly or straight needles can alter results)
- Post-procurement issues such as time between collection and analysis and storage method (noted most work done with cryopreserved PBMCS)
- Access to control samples: validated healthy controls are rare, definition of healthy can vary (medication status, exercise, age, etc.), there is a need for randomization and there are issues of ethics approval to consider

1.4.4 Issues for data acquisition, analysis, and reporting

- A need for distinct standardization for fresh vs. frozen samples
- Need for general gating strategies depending on complexity of panel and nature of sample: dead and viable cells, doublet discrimination, FSC vs. SSC, and determining of background and types of controls required including activation status of cells (Flow Minus One or isotype control antibodies)
- Automated data analysis
- Statistical consideration: how many events are necessary?
- How to report quantified data?

1.4.5 Data management and storage

- Standardize ways to name the data files and filing software
- How to storage and manage data in small/large studies?
- How to integrate data?
- Post-acquisition re-analysis of samples for ongoing and future studies

E.1.5. Funding

The possibility of applying to various governmental sources of funding would be difficult, particularly in the current context. Support of infrastructure alone, and its operation, is not a feasible option. The funding of such initiative would need to be incorporated in the context of various “disease-specific” initiatives. If funding is secured, it must be used well and not diverted. It needs to be tagged to specific projects overseen by Consortium.

E.1.6. Recommendations and future action plans

- Undergo extensive environmental scan of what has been done elsewhere in terms of human immunophenotyping. The group reiterated the importance of not "reinventing the wheel" as several other initiatives and consortia around the world have already established robust and applicable guidelines in multiple areas of investigation.
- Establish a national repository for SOPs and multi-marker panels.
  - Pre-analytical variables: Sample procurement, storage and preparation
  - Panel design
- Recommend universal guidelines instead of a professional obligation
  - In unison with professional associations
  - Sensitize the community to be compliant: compliance is real bottleneck
  - Establishment of Canadian Guidelines for Flow cytometry
- Training sessions and workshops: satellite to CSI or FOCIS, or workshop/scientific symposium
- Webinar series on various aspects of flow cytometry
  - Crosstalk with companies (US and CDN)
- Centralized report system where users can actively interact online
  - "Immuno-blog" (Resources for asking questions)

E.2 Theme 2 – *Panels and SOPs*

E.2.1 Recognizing the Need

Across Canada, approaches to harmonize methodologies including flow cytometry panels to maximize quality and impact of data are lacking. There is a complex landscape because multiple distinct constituencies exist for whom standardization through SOPs and common/overlapping panels would be of interest, however specific needs/priorities/mechanisms of support differ. The range includes:

- Academic research labs studying basic human immunology
- Research at the clinical interface: (e.g. organized cohort sample sets)
- Transitioning from research to clinical use (biomarkers/diagnostics)
- Clinical Diagnostics (CLIA certified; health care supported)

There is a major distinction between the needs/approaches/priorities for standardizing human immune monitoring in the clinical laboratory context versus the academic research context. Activities at the interface (clinical-research) typically represent a hybrid.

E.2.2. Harmonization versus Standardization

The group discussed the pros and cons of Standardization vs. Harmonization. Strict standardization (using SOPs) designed to ensure identical approaches are utilized, are distinguished from harmonization, which strives to ensure as much consistency as practically possible, without insisting on identical approaches. To find a happy medium, standardization and harmonization need not be mutually exclusive or in competition with each other. A more attainable approach would be to maximize those elements that can be fully standardized (using SOPs), while defining the range of acceptable practices that would not be identical, but *sufficiently similar* that overall harmonization is achieved resulting in Harmonized Operating Procedures (HOP).

For certain applications, a practical approach may be 'staging' - initially aim to harmonize and later re-assess potential to standardize.

The group emphasized that major strengths exist and it is important not reinvent wheels! There is much to be learned from existing experience and resources (see Theme 1).

E.2.3. Defining the SOPs that are needed

Specific Variables for Harmonization:

- Sample Type (e.g. whole blood vs. PBMC)
- Sample quality (how to ensure quality control/QC)
- Procurement/Handling/Freezing/thawing – pre-analytical
- Panels themselves (starting with reagents and controls)
- Machine set up
- Approach to Analysis of raw data
- Define Parameters of use (eg 10% variation acceptable)
- 'SOP of SOP': Defining when ready to be SOP? When to update?

Not a 'one-fit-for-all-purposes': it is apparent that multiple different approaches (SOPs, panels) are currently in use. Design as well as implementation of SOPs differs based on a range of considerations that can be: study-specific, question-driven, and requiring context-specific validation. For at least some programs, a degree of flexibility is required for setting up and adapting panels. Two general but not mutually exclusive approaches were suggested.

1. Building a Library SOPs: a common repository of SOPs/HOPs would enable communities to identify most suitable 'fit-for-purpose' approach that could still harmonize with other initiatives (better than entirely independent approach). Particularly useful for those starting out without already-defined approach

2. 'Core-based' SOP/HOP for flow panels: establishing a common 'backbone' for panels that incorporates common elements likely of broad use, upon which 'fit-for-purpose' additions can be made.

E.2.4 Obstacles

A number of key issues were identified that pose challenges to future harmonizing efforts: 1. Achieving the balance between SOP stringency and broader utility. 2. Imposing additional cost for researchers is a major issue. There was a suggestion that this could be supported for clinical studies but not likely feasible everywhere. 3. Clinical labs: though substantial capabilities and key ingredients exist such as technical expertise, infrastructure and patient interface, these strengths are typically not accessible for research purposes. The group wondered if this could be changed. 4. There is a lack of standards even for elements of SOP/HOP (i.e. of actual measurements; comprehensive database; etc.). 5. We still need a 'needs analysis' to better define hurdles and bottlenecks.

E.2.5 Recommendations for deliverables for CHIC

1. 'Yes' to Consortium, to continue discussion to help harmonize/enhance utility/efficiency of clinical and research missions.
2. Establish National Library of validated SOP/HOP/panels for community to access: (Library should be a resource, not viewed as formal expert advice, and not meant to be proscriptive or mandated, rather sharing of experience). Next steps to include:
   - Start relatively simple: e.g. blood draws, freeze/thaw approach prior to more complex SOP/HOP
   - Solicit 'SOP/HOP's; compiling/categorizing; identifying context
   - Soliciting community's experience with encountered/addressed problems
   - Requires mechanism to decide whether SOP/HOP is ready to include in library? Approach (if any) to updating?
3. Sample sharing program: comparing platforms and infrastructure

4. Generate a list of hurdles/bottlenecks/current needs AND share with industry, other experienced partners to become informed of existing solutions
5. Establish creative partnerships with Industry, CIHR and clinical areas.
6. Take advantage of clinical lab expertise/infrastructure/patient interface: add a research-dedicated machine into clinical labs
7. There is the potential to identify a particular 'project' focus to drive the success of CHIC harmonization efforts?

E.3 Theme 3 - *Big Data – Repository and Management*

The group had representation from Principle investigators, core managers, industry representation (IBM, BD), NRC, and trainees. The group explored what is in current use, what is needed and useful and ideas for what is possible. The group was asked to consider the issues of how to handle the large datasets that are generated, ensure robustness of annotation, have trusted automated processes for high-throughput analysis and satisfy funding agency expectations of data-sharing.

E.3.1 Need

A data repository would be a valuable resource for future. Establishing normal healthy reference was a priority that would benefit the whole community. Subjectivity in data analysis is a major problem for multi-center trials, and for comparing one study to another. However, there is skepticism about automated analysis tools and this needs to be addressed through education and experience. There is a large gap in individuals trained in analyzing multidimensional data (flow informatics). Flow is the obvious first area to examine, but integrating with newer techniques with even higher complexity such as Cytof is required.

E.3.2 Current Practices and Platforms

Some large centres have access to cloud based storage because they are using particular software (e.g. SickKids in Toronto has Cytobank). There are many examples of how to build public databases from the *omics* fields (genomics, microarray, etc.) and we have experts in the group from genomics. There are commercial options for automated data analysis and some homegrown platforms and each have strength and weaknesses and they are evolving rapidly. There are shifts to using population finding algorithms and unsupervised cluster analysis (SPADE etc.) to use a more systematic approach. There is a need for "dimensional reduction". Ryan Brinkman (BCCRC) is a Canadian at the forefront of developing flow informatics and part of FlowCAP Project - Flow Cytometry: Critical Assessment of

Population Identification Methods. The typical level of data annotation is a major shortcoming even in current publications despite ISAC recommendations for minimal reporting. Ryan has agreed to join the CHIC steering committee.

E.3.3 Issues discussed included:

The need for quality control on datasets in a repository and how concepts of harmonized and standardized apply to collection of the data prior to its deposition is an important issue. How will quality control be done? Could sites be certified in some way to participate to ensure QC of site? The standards for deposition would need to be defined. Can they evolve once set?

There may be ethical issues in depositing certain datasets, but if appropriately de-identified it should be fine similar to other public databases with human samples.

Would funding agencies help to promote the harmonization by requiring deposition in a public repository?

The group envisioned two possible scenarios:

1) *a fully public repository supported by some analysis tools, or*
2) *a closed one that only members could access but might also provide access to more elaborate analysis tools (modeled on how Cytobank works but it would be buying licenses or developing in house tools).*

Anticipating the type of metadata to tag might be hard to agree on, as it is a moving target as technology changes and it is hard to anticipate future needs.

High performance computing and storage are widely available. Organizations such as Compute Canada may be good partners. The biggest gap is the lack of trained individuals who understand the biology and can apply and innovate the automated analysis tools.

It would be easier if journals enforced some standards for reporting of flow data and this may be coming.

E.3.4 What CHIC could accomplish in this area.

The key recommendations for a Data Repository is that it would provide infrastructure, oversight and support, be able to enforce minimum standards for

annotation to maximize the usefulness of the datasets for comparative or meta-analysis, demonstrate a culture of sharing which would together raise clout with funding agencies for future funding initiatives (particularly large network type grants that would not need to reinvent the wheel). The ability to scrutinize and use raw data can reveal issues of compensation that may skew results.

For Data Analysis, it was suggested that centres of the consortium could play a leading role in innovation and training. There could be training for established tools through workshops tagged to conferences or webinars. This is particularly helpful as the field is moving so quickly. Partnerships to develop and share new tools would be facilitated.

The consortium could provide consulting in the flow-informatics area.

There was considerable enthusiasm that a consortium could develop a national training program in "*flow informatics*" to address the large gap in HQP in the area.

There are many opportunities to partner with government agencies, biotech, pharma and computing industries for the infrastructure and support, innovation in tools and sponsoring of training programs.

Industry might be able to provide data for "healthy controls" from clinical trials more readily than for patients due to proprietary considerations.[1]

E.3.5 Goals/actionable items

Short term – establish a working group with broad expertise
Medium term – detailed environmental scan of current platforms, develop relationships with partners, build vision of infrastructure, quality control, training program and buy-in from the key players in establishment of a national data base.
Long term: 1. Establish funding, ongoing support and oversight of infrastructure,
2. Establish standards for depositing of datasets
3. Work on a national "normal" dataset
4. Develop national training program in flow-informatics

[1] For those in the know, if a trial is publically funded in part, is the data supposed to end up in the public domain?

5. Innovate new tools

Points raised in the full group discussion were:

- the importance of bioinformaticians/system biologists at the interface;
- data storage is now relatively inexpensive so that it should not be a barrier to a database
- concerns surrounded security of the database and whether there would be participation of groups, as some would want to keep their data private
- an attractive goal would be to harness health care to individual patients while concurrently generating research grade samples (a system in the UK where providing samples for research was integrated into the health system was given as example).
- being able to stratify on treatments would likely increase pharma's interest in supporting project

the importance of involving Ryan Brinkman was reiterated

Action items:

- - approach Ryan Brinkman for high-level involvement particularly for development of training program *. Although Ryan was unable to attend the workshop, he has agreed to join the CHIC interim steering committee.

- When a working group forms around the topic - continue dialogue with potential partners and funding agencies to develop vision of their role.

E.4 Theme 4- *Establishment of a national committee*

We asked whether there is a need for a national committee, what's its mandate should be, how it should be structured and function and how would it be funded. As well as what the short and long term goals should be**.** Here we summarize the comments from the attendees.

E.4.1 The Need.

With respect to the need for a committee, it was felt that there is a need to provide links between existing groups conducting similar exercises and bridging different silos. A repository for SOPs and panels and best practices would be useful. There is a need for standardization across fields. People in research labs, particularly those entering into human immunology field from other areas could benefit greatly from a reliable source of SOPs and standards. Soon journals will begin to mandate standards. The overall goal would be to establish specific guidelines for best

practices, including guidelines and SOPs for different kinds of samples, such as frozen cells and fresh blood. The long-term goal is to become a resource for human immunology research, to provide information on a standard human phenotype and provide guidelines for conduct of such research. There was discussion as to whether the committee should also become a data repository. There is a need for integration of human immunophenotyping data across diseases and databases and to be able to access and possibly re-gate data from published studies.

E.4.2 Mandate

There was discussion that groups exist for clinical immunology and how would CHIC national committee fit into such a picture. The group felt the focus should be on providing information to research groups. While there was some interest in providing SOPs, the SOPs should be flexible. Previous approaches have taken basic research approaches and moved them to the clinic. There was discussion as to whether assays should be standardized in the clinical realm and then taken back to the basic lab research- bedside to bench. Starting off with clinical could prove beneficial. In this regard, its worth noting that in Sweden, 6 clinical centers use the HIPC panel and that in Germany, insurance companies are paying for 150 immunodeficiency patients to be tested with the HIPC panel per year and the European consortium has now collected 300 healthy donor immunophenotypes using this protocol (communicated by Thomas Giese- FCE call Oct 2015). However, from the research point of view, the 8-colour HIPC panel may be too restrictive.

A national committee could be helpful in assimilating information and keeping track of efforts made by other groups. If international standards were established through FOCIS, a national committee would help to link Canadian researchers to these efforts. The emphasis at least initially should be on information gathering and distribution rather than enforcement of standards. Enforcement would be far more costly/difficult than having an information gathering/dissemination committee. It would be critical that the information provided is readily accessible and continually updated. Information provided must be evidence based. It was discussed whether our long-term goal will ultimately be to achieve certification.

An important **philosophical question discussed** is whether we should take the route used by the European group, ENTIRE, as outlined by Winqvist, in which they have harmonized/standardized human immunology in the clinic based on the HIPC panel and are using this panel to collect healthy donor reference data or whether we should come at this process from an academic point of view, with provision of more general and less prescriptive guidelines. Perhaps both could be

done by providing a set of standards to be used in clinical human immunophenotyping and coordinate closely with the Canadian Society for Allergy and Clinical Immunology (CSACI) and providing a set of best practices, core SOPs and general guidelines for academic research.

E.4.3 Process

It is important to link any National committee to existing groups, including FOCIS. There are already groups for clinical immunology- how would we link to these. There was discussion as to whether we should create CHIC centers that would operate on standard panels/SOPs or have a central repository that simply provides information to all users about best practices. There was general agreement that it is essential that such a repository for SOPs and best practices be kept continually up to date in order to be relevant and broken down for different subfields or disease. It was felt that it would be difficult to have an enforcement role. Existing groups focus on clinical standards. Instead, the information provided by a consortium needs to be enticing to make people want to use the standardized protocols and needs to be readily accessible and kept up to date. Key questions that were raised are how to ensure that labs can follow the SOPs, and should we eventually move to certification?

E.4.4 Funding

Sources of funding for a consortium will be difficult to secure in Canada. We could access government agencies, funding agencies, existing NCEs, private sector. However, we cannot endorse specific technologies to get funding. The CFI has a Cyber infrastructure competition and we might fit this concept (NOI due April 2016 and full proposals due October 2016). Perhaps working around automated gating and big data storage on human immunophenotyping.

E.4.5 Obstacles and Issues.

There is a need to continually update. Integration of information from different groups needs validating. Moreover, there will still be a need for validation of assays. Funding is an issue in order to keep the process going. Establishing SOPs and panels without endorsing one reagent and yet still getting industry buy in is a critical issue. Getting people to follow SOPs and accept the standards is another key issue.

E.4.6 Short to midterm term goals.

1. Form a committee:

It was felt that we need an initial committee of enthusiastic volunteers, who will start the process and establish some credibility before applying for funding. The initial working committee should have representation from different disease areas, such as cancer, transplant, HIV and should have diverse representation including both clinical and academic researchers with regional representation. In terms of process, the suggestion was made to send out a wide call for volunteers to a range of interest groups and have an open recruitment process. An alternative view was to start with a selected committee of the willing from the attendees at the workshop and initiate a broader consultation process when forming subcommittees. It was felt that the initial committee should consult broadly with existing groups and should also forge links to national and international immunology community through Canadian Society for Immunology, the Canadian Society for Allergy and Clinical Immunology (CSACI) and FOCIS.

2. Start with Information gathering: The initial goal of this committee should be to start an information gathering/scanning process. We can send out a call to Immunologists for SOPs and panels, recognizing that SOPs and panels may be domain specific. However, basics like cell collection/freezing should apply to everyone. For example, CTRnet already has SOPs for tissue collection and storage. It will be important to evaluate lessons learned from other efforts of this type (successes and failures). Both clinical and academic needs should be considered.

3. Filter the evidence. After information gathering, it will be important to filter the evidence, although how this will be done was not discussed in detail. However, it should be evidence based and consolidate existing standardization efforts. There is a need to rank and compare existing protocols. It was discussed that this will be a significant undertaking and whether we need to do this professionally or have students undertake this as a project.

4. Synthesizing information across fields. Having done a scan of the environment and done some filtering it will be important to synthesize the information across fields and make it available through a web site.

5. Determine funding sources. Fund raising will need to start fairly soon, but could be a goal after we establish more clearly our initial plan and start the information gathering process.

E.4.7 Long-term goals

1) Become a research resource for SOPs and Best practices. The long-term goals of this initiative are to become a resource for research by providing a set of established guidelines and best practices as well as SOPs. These should be made available to the community through a well maintained website through regular updating and an interactive process where feedback and corrections can be incorporated.

2) Consolidate data on what is the normal immune system. We would like to be able to collate information on the definition of normal immune systems and make those reference data available and ultimately provide a source of information on how this state changes with specific diseases.

3) *Develop a program of outreach and training.* Provide training on best practices in human immunophenotyping. Ultimately, we might move towards providing a certification system, indicating that a center is CHIC compliant.

4) If feasible, *provide access to a/form a data repository* where people can access published data on human immunophenotyping such that data can be reanalyzed with alternate gating

5) *Integrate human immunophenotyping data with other national databases*.

E.4.8 Action items:

Create an interim steering committee
Make contact with relevant stakeholders and begin gathering information
Define a process for vetting and renewing the committee
Define a process for gathering and vetting information
Establish a funding model

Groups mentioned as relevant to this effort:

FOCIS
Canadian Society for Immunology (CSI)
Canadian Society for Allergy and Clinical Immunology (CSACI)
Related NCE (Many possible groups- Allergen…)
Canadian Cancer Immunotherapy Consortium
CNTRP (transplant)
CCMA (cytometry and microscopy)
ICCS (clinical society)
CTN

CIRN – (Canadian immunization Research Network)
Canadian Partnership for Tomorrow - Cohort being followed for last 20 years with clinical markers

F. PLENARY SESSION - RECOMMENDATIONS

F.1 The Need.

The community would welcome an information resource/repository for researchers that provides access to best practices and links to existing studies on the human immunophenotype. The short-term goal should be information gathering and distribution rather than enforcement. It will be important to keep track of efforts made by other groups and make these available through a website and to gather information in one place, from groups already undertaking similar exercises for their sector. Where data exists, CHIC should evaluate lessons learned from similar attempts at harmonization.

F.2 Challenges and Obstacles.

We can learn from clinical laboratories about standardization, but as pointed out by our keynote speaker, standardization is a very high bar- perhaps focusing on information gathering, filtering and dissemination and harmonization of methods and approaches is more realistic. Enforcement is unrealistic at this stage. A key issue is how to filter the information. For example, how can we compare SOPs and ultimately choose some. The information gathering, filtering and dissemination process will be labour intensive. How do we fund the initiative so that it is done systematically and professionally and how to maintain a relevant site that people will respect and use is the a key challenge.

F.3 Goals:

1. Form a working committee of the enthusiastic willing- keep it small, but of sufficient breadth in disease focus, geographical distribution and expertise, including clinical, academic and technical.
2. Working committee makes contact with stakeholders regarding the needs and willingness to harmonize protocols and makes a philosophical decision on clinical first or basic first or both.
3. Begin a process of environmental scanning. Contact groups with call for SOPs and information. Consult industry and clinical immunology community about their standards and expertise. Scan the literature for similar studies.
4. Establish a process for filtering the information
5. Synthesize protocols across fields where possible

6. Website creation
7. Outreach and training
8. Determine funding sources: Government agencies, private sector, NCEs

F.4 Recommendations:

Based on the attendance and comments at the meeting, we believe that there is strong support for formation of a national committee, the Canadian Human Immunology Consortium, CHIC, that will develop a human immunophenotyping research resource, that will scan the environment and synthesize a set of guidelines, best practices and SOPs for conducting human immunology research. The committee will start with information gathering and forming links to existing groups nationally and internationally and will endeavor to be inclusive of different research areas in human immunology, with input from basic and clinical scientists and with regional representation.

F.5 Action items:

1. **Form a working committee.** The first step in the process is to form a working committee. Two suggestions have been discussed. The first is to send out a call to various stakeholders across Canada to ask for volunteers and the second is to choose a committee of the enthusiastic willing from the CHIC organizing committee supplemented by participants from the meeting representing different clinical areas, particularly focusing on a few key people already involved or experienced in national harmonization efforts in their sector. We suggest no more than 8-10 for the initial steering committee. Once established, the interim steering committee would establish a process for vetting and renewing the committee and would also define the tasks ahead and any subcommittees needed to achieve those tasks.
2. **Plan a series of phone calls and face- face meetings of the interim steering committee** (perhaps in conjunction with CSI and FOCIS meetings) so the working group can discuss these tasks efficiently. Recommendation from Ola Winqvist is that face-face meetings essential. First meetings to establish philosophy and action plan.
3. **Find a source of funds to hire a project manager** to begin the collection of data. Consider applying for April 2016 NOI for CFI cyberinfrastructure.
4. **Start the environmental scan:** Contact stakeholders regarding their interest in such a national information repository/resource for human immunology; Contact groups already doing immunophenotyping for their best practices and SOPs; Scan the literature for already published efforts and evaluate successes and failures.

5. **Develop a working plan** to systematically collect and review the data and begin the process of forming a website to disseminate the information.
6. **Form subcommittees**, bringing in additional members from the community: Funding committee, education committee, an SOP and best practices committee, a Data analysis committee to begin to put together information and events to further the development of CHIC.
7. **Develop the CHIC website.** This can wait for some information to be gathered, but could initially be a shell indicating the process and where to send information for our systematic review.

G. FINAL CONCLUSIONS OF SYMPOSIUM

As outlined above, there was strong support among the attendees of CHIC that a working committee be formed initially by the organizers of the Sept. 26 symposium, to begin the process of forming a national committee to recommend best practices for conducting and reporting multiparameter human immunophenotyping studies. The organizing committee has invited 4 additional members to join to form an initial working committee of 8 members and will move towards consulting the community further and defining the structure and governance of the working committee.

H. POST-SYMPOSIUM UPCOMING STEPS

1. Circulate the report to the attendees of the first CHIC symposium for feedback.
2. Arrange a series of phone calls and face-to-face meetings of the initial working committee to:
- identify an interim leader and mandate of the initial steering group
- define the process for defining the structure and governance of CHIC
- consult the community
- finding a source of funds to hire a project manager

I. NO CONFLICT DISCLOSURE

The writers of this report declare that they have no conflict of interest to declare with respect to the opinions presented in this report.

Signed: on December 26, 2015

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