

MCW Cancer Center Cell Therapy Laboratory Shared Resource

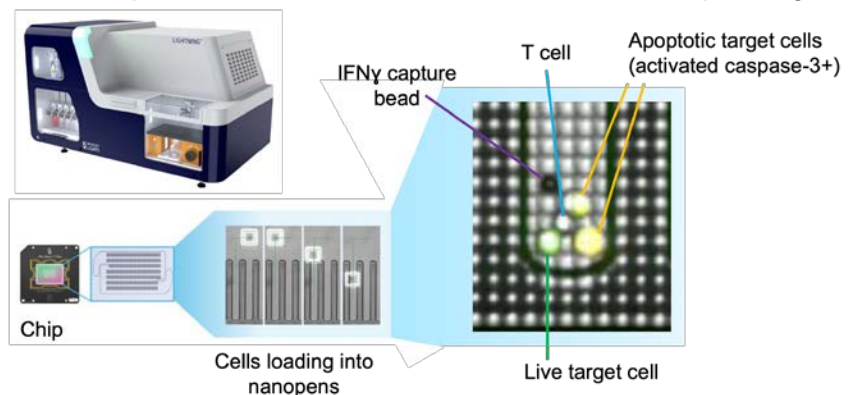
The Cell Therapy Laboratory (CTL) focus on cellular therapy to improve outcomes of blood and marrow transplantation and other cell therapies to combat human cancers. Its main objectives are to provide expert consultation to investigators for the translation of cellular therapies to the clinic, to provide cutting-edge instrumentation needed to support the development of novel cell therapy strategies, and to provide a facility suitable for the processing and production of cellular therapy products for clinical use.

The CTL is a shared resource supported by the MCW Cancer Center and Froedtert Hospital to provide advanced cell therapy and immune monitoring services across the MCW campus. The CTL Laboratory consists of multiple clinical and research lab spaces that are all under the guidance of Bryon Johnson, PhD, Laboratory Director and Tyce Kearl, MD, PhD, Research Director.

- (a) Cell Processing Laboratory (CPL), where all hematopoietic cell and blood cell processing activities for the combined adult/pediatric Blood and Marrow Transplant Program occur.
- (b) Lymphocyte Propagation Laboratory (LPL), where cell processing and manufacturing done under the guidance of FDA INDs has been conducted. We are also available to help investigators develop novel cell therapies for clinical trials. The space is used for in-house CAR-T cell manufacturing using CliniMACS Prodigy devices, other cell manufacturing processes that involve gene vectors, and use of the CliniMACS Prodigy devices for cytokine capture of virus-specific T cells.
- (c) Immune Monitoring Lab (IML), where cell products, patient biospecimens, and other research samples are tested using various immune profiling assays. Highlights of the offered services include:
 - Single-cell functional, phenotypic, and genomic assays using the Berkeley Lights Lightning Platform
 - Single-cell and bulk cytokine & biomarker assays using the IsoPlexis (a chip-based technology that allows single-cell analysis of more than 30 cytokines simultaneously) and Luminex Flexmap 3D platforms (multiplex assays)
 - Immunophenotyping using flow cytometry (MACS Quant 10 flow cytometer; 8-colors)
 - Cell cytotoxicity assays using an xCelligence real time cell analysis (RTCA) instrument.

Berkely Lights Lightning Optofluidics Platform: This is a novel optofluidic platform that allows one to visualize the phenotype & function of **individual cells** on a microchip that contains 1500 chambers. Cells & other particles can be moved within the chip to place them in selected chambers. Patented light technology applies a non-destructive visible light to the chip substrate with creates a light-induced electric field that “moves” the cells/particles and places them in selected nano chambers. Depending on your cell size, the chambers can hold up to 30 cells each.

A major strength of the system is that you can recover the cells of interest for further characterization. You are able to upload any single nano chamber on the chip for export into a single well for downstream analysis (genomic analysis, additional functional analyses, etc.).

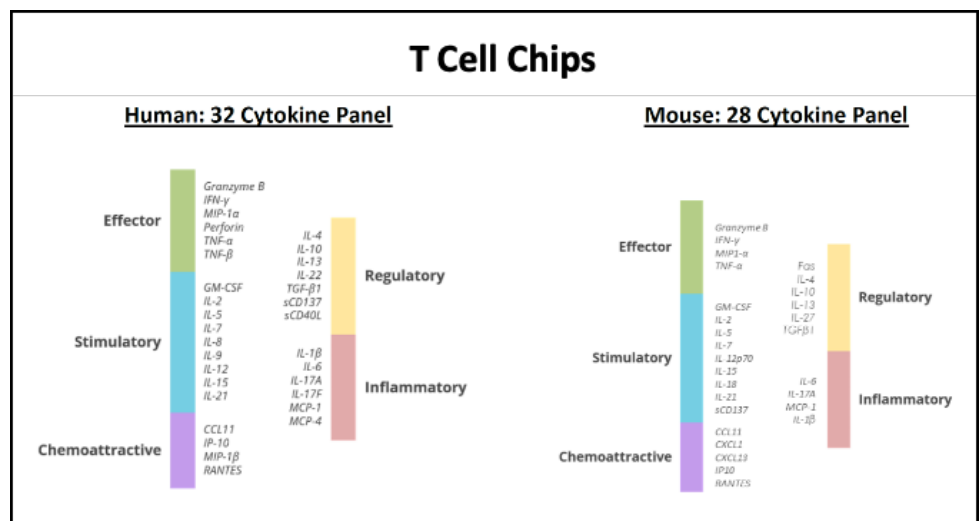


Importantly, the system has a large number of capabilities and the company is continuing to develop new workflows; one of the current workflows involves 'Cell Therapy Development'.

Isoplexis (can be used for both human and mouse): This chip-based platform analyzes approximately 2000 single cells (T cell chip) for the production of 32 human cytokines or 28 mouse cytokines and effector molecules including effector, stimulatory, chemoattractive, regulatory and inflammatory mediators. The analysis process generates a 'polyfunctional stimulation index' (PSI) which is defined as the percentage of polyfunctional single cells (*i.e.*, secreting 2 or more proteins) in a sample, multiplied by the average signal intensity of the secreted proteins in individual functional groups (effector, stimulatory, chemoattractive, regulatory, inflammatory) from each cell. High frequencies of polyfunctional T cells have been previously associated with cancer vaccine-induced efficacy in murine cancer models (Auricchio *et al.*, 2019). Notably, it has recently been shown that the PSI of pre-infusion CD19 chimeric antigen receptor (CAR)-engineered T cells is associated with post-infusion clinical response in non-Hodgkin lymphoma patients (Rossi *et al.*, 2018), highlighting the power of the PSI analysis.



Briefly, to do the assays T cells (total CD3 cells, or separate CD4 & CD8 subsets) are stimulated in culture overnight with a source of antigen or polyclonal activator, and then loaded onto the chips. The chips are then read for cytokine production over a period of 20-24 additional hours. In addition to generating PSI values, another advantage of the Isoplexis system is its ability to provide information on the secretion of each individual cytokine at the single cell level.



Isoplexis also has a 'Single-Cell Innate Immune' cell chip, and several other chips are in development (see updates and protocols at Isoplexis.com).

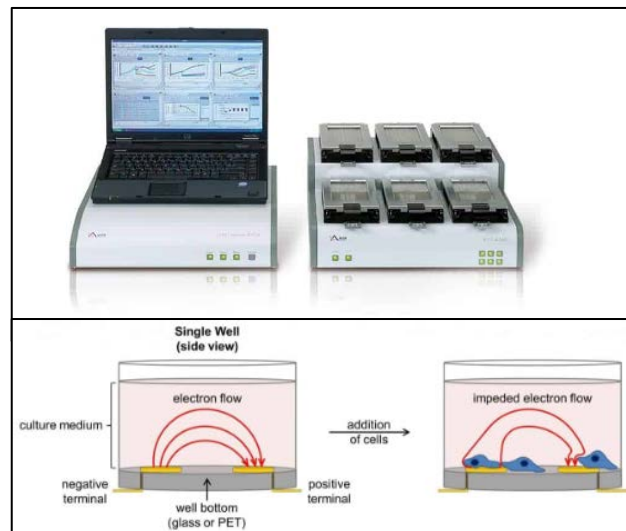
Luminex Flexmap 3D: The FLEXMAP 3D[®] system combines differentially dyed fluorescent microsphere sets with an innovative instrument design to enable precise, flexible, rapid multiplexing of up to 500 unique assays within a single sample. This instrument can take a 96 or 384-well plate format. More than 40 analytes can be examined in a single sample using a maximum of 25 μ l. Several analyte panels are available or custom panels can be designed, and the instrument has 4.5 logs dynamic range.



MACSQuant 10 Flow Cytometer: This is a 10-parameter, 8-color flow cytometry analyzer manufactured by Miltenyi Biotec. This instrument is available for analysis of clinical samples. One can design their own flow cytometry panels or use a CAR T Cell Express Mode Package that contains the panels shown in the attached table. Express Modes are a unique add-on for the MACSQuantify Software, developed to simplify flow cytometric analysis (see attachment below). They automate the measurement and analysis of flow experiments via predefined experiment settings as well as acquisition and automated analysis. A gating of flow cytometry results will be automatically adjusted for each data file individually to achieve optimal results. Using the Express Modes simplifies flow cytometric analysis and ensures reproducible data analysis.



xCelligence RTCA 6-Plate System: This instrument can allow for measurement of immune cell cytotoxicity or cell death in a 96-well format (maximum of 6 plates at one time). Importantly, the system is label free, real-time, and assays can be performed over several hours-days. Target cells need to either be plastic adherent or can be tethered to the well surface using plate-bound antibodies against a cell-surface antigen present on the target cells. The system is based on electrical impedance. Adhesion of cells to gold microelectrodes in each well impedes the flow of electric current between electrodes (see diagram on the right). This impedance value, plotted as a unitless parameter called “Cell Index”, increases as cells proliferate and then plateaus as cells approach 100% confluence. If immune effector cells or a drug induces the destruction of the target adherent cells, the corresponding cytolytic activity can be sensitively and precisely detected. The continuous acquisition of impedance data for each well enables the generation of real-time killing curves for multiple conditions simultaneously. The system contains an integrated software package that includes a powerful immunotherapy module for running and analyzing real time cell analysis data.



Three of the spaces are located on the 3rd floor of the Froedtert Hospital (FH) Pavilion building; the other space is located on the 5th floor of the Froedtert & MCW Clinical Cancer Center building. Lab spaces in the FH Pavilion (Suite 302-324) contain the following: The space houses a total of 4 CliniMACS Prodigy devices.

The Cell Therapy Laboratory is fully equipped for cell processing, cell culture, and cell production and storage. Key equipment includes incubators, biological safety cabinets, centrifuges, microscopes,

hematology analyzers, refrigerators, freezers, sterile connecting devices, and heat sealers. The IML space contains equipment needed for assays of cell proliferation, ELISPOT, ELISA, PCR (droplet digital system and standard PCR), cell cytotoxicity, single-cell analyses (Berkeley Lights and Isoplexis platforms), and up to 8-color flow cytometry. The air in all lab spaces is hepa-filtered and positive pressure. Shared support rooms (approx. 3000 sq. ft.) include freezer space (LN₂, -80°C freezers), CO₂ gas storage and distribution, a storeroom, dedicated spaces for flow cytometry and molecular biology, along with clerical and office space.