APPLICATION NOTE

# IsoPlexis Proteomic Product Suite for Cancer Immunology

IsoPlexis' functional proteomics reveals unique secretomic signatures and insights into Cancer Immunology

**In this Application Note we outline:**

- Challenges in cancer immunology
- Understanding cancer cell communication to infer a strategy to inhibit tumor cell metastasis
- An inhibitory mechanism of monocytes toward immune suppression in Follicular Lymphoma (FL)
- Mechanisms of immune persistence in solid tumor indications
- Resolving tumor heterogeneity to reveal independent trajectories of drug tolerance



© 2020 IsoPlexis, Inc. FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

## Prep, Run, Analyze

**High Level Challenges and Applications**

**Application 1:** Secreted Cytokines in the Promotion of Tumor Metastasis

**Application 2:** Inhibitory Mechanism of Monocytes Toward Immune Suppression in Follicular Lymphoma (FL)

**Application 3:** Mechanisms of Immune Persistence in Solid Tumor Indications

**Application 4:** Resolving Tumor Heterogeneity to Reveal Independent Trajectories of Drug Tolerance

**IsoPlexis Product Types that Address These Challenges:**

- CodePlex Secretome
- Single-Cell Secretome (Human)
- Single-Cell Secretome (Mouse)
- Single-Cell Intracellular Proteome
- Single-Cell Metabolome

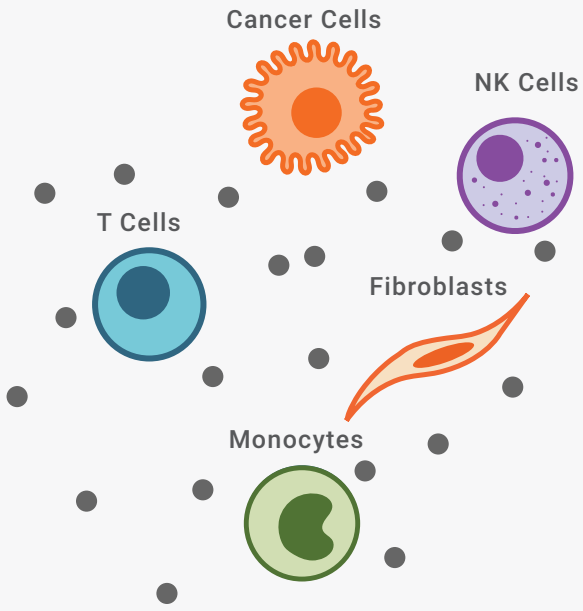
### Overcoming Challenges in Cancer Immunology

Cancer cells in the tumor microenvironment secrete cytokines that interact with other cells, such as immune cells, which facilitates intracellular communications and jointly moderates pathophysiological processes, including cancer-induced angiogenesis and metastasis. Secretomic profiles of cancer cells advance as they multiply and become more invasive, which suggests that secreted factors may be associated with proliferation and migration.

Heterogeneity in cytokine production and aberrant cytokine signatures can create challenges in cancer immunotherapy. IsoPlexis' platform is helping leaders accelerate their workflows by functionally defining each cell type to inform more effective therapies.

- **Challenge 1: Requires Highly Multiplexed CodePlex Secretome Solution**
- **Challenge 2: Requires Single-Cell Secretome and CodePlex Secretome Solutions**
- **Challenge 3: Requires Single-Cell Secretome Solution**
- **Challenge 4: Requires Single-Cell Intracellular Proteome and Single-Cell Metabolome Solutions**

### Cell Types and Cytokines Implicated




Functionally defining each cell type involved in the immune response in cancer immunology.

## Why Cell Subsets for Multiplexing Cytokines Matter in Cancer Immunology




Traditional technologies average serum protein information from all cells. IsoPlexis' cellullar functional phenotyping uncovers cellular differences to identify functional mechanisms in cancer immunology.

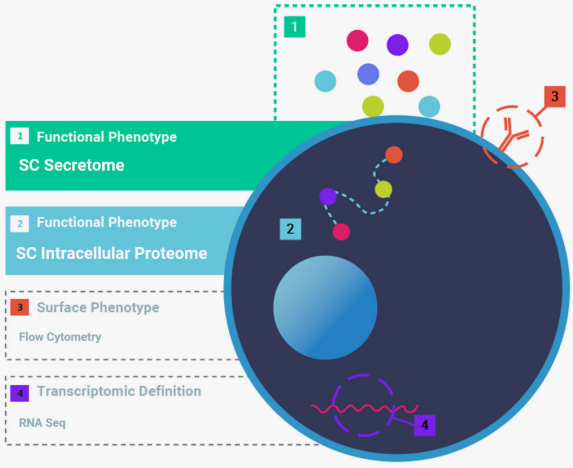
## Understanding Cellular Immune Function is Critical for Therapy Development in Cancer Immunology

Traditional technologies average serum protein information from all cells. In a variety of trials†, stratification of responders from non-responders is not possible with status quo technologies. Data shows that what specific cytokines are produced by each heterogenous immune cell matters, and IsoPlexis' cellullar functional phenotyping uncovers these cellular differences.

Through analysis of cellular RNA or surface phenotypes alone, you may be missing essential functional extracellular phenotypic differences that reveal the biological drivers of patient response. IsoPlexis' single-cell functional proteomics fills the existing gap in complete cellular characterization.

† isoplexis.com/publications

## Multiplexed Proteomic Characterization: Filling the Existing Gap in Full Cellular Characterization from Single-Cells




Through analysis of cellular RNA or surface phenotypes alone, functional extracellular and intracellular phenotypic differences that reveal the biological drivers of patient response may be missed.

# Prep, Run, Analyze

## Detecting Multiplexed Serum Protein from Ultra Low Sample Volume is Critical For Understanding Tumor and Immune Activity

The IsoLight is the only system that enables researchers to obtain highly multiplexed cytokine data without advanced training and without interaction with the samples. Furthermore, the IsoLight is also the only system to:

(1) Perform multiplexed proteomic detection of 20-40 cytokine markers simultaneously, to provide early predictive metrics of functional and inflammatory cytokines

(2) Provide an automated, all-in-one system, for increased work-away time

(3) Handle a smaller amount of sample volume if large blood draws are not possible, making it capable of handling a wider range of clinical sample sizes

By functionally defining each cell type involved in the immune response, researchers can better understand the functional mechanisms for the development of patient biomarkers and novel therapies in cancer immunology.

## CodePlex Secretome Panels

### Panel Menu

Granzyme B, IFN-y, MIP-1a, Perforin, TNF-a, TNF-B, GM-CSF, IL-2, IL-5, IL-7, IL-8, IL-9, IL-12, IL-15, IL-21, CCL11, IP-10, MIP-1B, RANTES, IL-4, IL-10, IL-13, IL-22, TGF-B1, sCD137, sCD40L, IL-1B, IL-6, IL-17a, IL17F, MCP1, MCP-4, IL-18, TGF-a, BCA-1, IL-12-p40, MIF, EGF, PDGF-BB, VEGF

### Human Adaptive Immune

IL-17A, TGF-b1, MIP-1a, IL-9, MIP-1b, IL-6, IL-7, IL-8, IFN-y, IP-10, GM-CSF, IL-4, IL-5, IL-10, TNF-a, MCP-1, IL-13, IL-2, Perforin, sCD137, TNF-b, Granzyme B, IL-15

### Human Innate Immune

IL-17A, TGF-b1, MIP-1a, MIP-1b, IL-6, IL-7, IL-8, IFN-y, IP-10, GM-CSF, IL-4, IL-5, IL-10, TNF-a, MCP-1, IL-2, Perforin, sCD40L, sCD137, TNF-b, Granzyme B, IL-15, PDGF-BB

### Human Cytokine Storm Panel

IL-1b, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-13, IL-17, MCP-1, GM-CSF, MIP-1a, MIP-1b, TNF-a, IFN-y

### Stem Cell Signaling

IL-17A, TGF-b1, MIP-1a, MIP-1b, IL-6, IL-8, IFN-y, GM-CSF, IL-4, IL-10, TNF-a, MCP-1, IL-2, IL-15, Rantes (MPN), IL1a, IL1b, IL12, CCL2, CXCL5 * (MPN)

### Cancer Signaling

IL-6, IL-7, IFN-y, IL-4, IL-5, IL-10, TNF-a, MCP-1, IL-13, IL-2, EGF, PDGF-BB, VEGF, Rantes (MPN), MIF, FGF, HGF, IL1a, IL1b, IL12

| Status Quo Multiplexed Bulk Analysis |
|---|
| X Up to 100-200 uL per sample (for replicates) |
| X 6-10 hours of hands-on sample prep time |
| X Workflow requires multiple steps and user interaction points |
| X Fill 96 samples before run |
| X Multiple systems required to generate and analyze data |
| X Limit of Detection: 5-5000 pg/ml |
| X Data analysis and visualizations require much user input and are not automated |

| CodePlex Secretome |
|---|
| ✓ 11 uL per samples (for replicates) |
| ✓ 5 minutes of hands-on time |
| ✓ Completely automated workflow |
| ✓ Modular, load 8-64 samples per run |
| ✓ One system: The IsoLight |
| ✓ Limit of Detection: 5-5000 pg/ml |
| ✓ State-of-the-art data analysis software with advanced visualizations |

The CodePlex Secretome Solution measures 30+ cytokines in bulk, automated on the IsoLight system, and can selectively run eight conditions a chip in “MacroChambers” across eight chips on a single run. Easily run replicates with a small sample volume: 5.5 uL per microwell (11 uL per sample replicate).

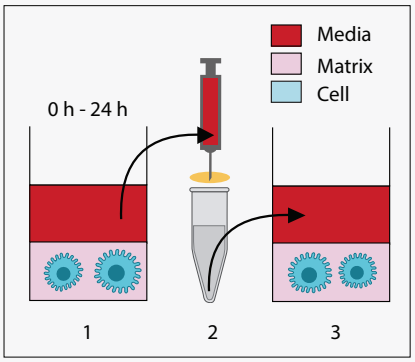
## Application 1 – Understanding Cancer Cell Communication to Infer a Strategy to Inhibit Tumor Cell Metastasis

*IsoPlexis' Functional Proteomics Reveals Unique Synergistic Paracrine Signaling Pathway Promoting Cell Migration*

**Products Used**

CodePlex Secretome

### Secreted Cytokines Promote Tumor Metastasis




| | HT1080WT | | | | | | |
|---|---|---|---|---|---|---|---|
| | 10 | 20 | 50 | 100 | 120 | 150 | $R^2$ |
| EGF | | | | | | | 0.07 |
| FGF | | | | | | | 0.01 |
| HGF | | | | | | | 0.01 |
| VEGF | | | | | | | 0.40 |
| PDGF | | | | | | | 0.02 |
| MIF | | | | | | | 0.82 |
| MCP1 | | | | | | | 0.09 |
| RANTES | | | | | | | 0.57 |
| MIP1a | | | | | | | 0.01 |
| IFNg | | | | | | | 0.17 |
| TNFa | | | | | | | 0.22 |
| TNFb | | | | | | | 0.68 |
| GMCSF | | | | | | | 0.01 |
| IL1a | | | | | | | 0.17 |
| IL1b | | | | | | | 0.10 |
| IL2 | | | | | | | 0.09 |
| IL4 | | | | | | | 0.14 |
| IL5 | | | | | | | 0.34 |
| IL6 | | | | | | | 0.85 |
| IL8 | | | | | | | 0.83 |
| IL10 | | | | | | | 0.00 |
| IL12 | | | | | | | 0.03 |
| IL13 | | | | | | | 0.25 |
| FITC | | | | | | | 0.41 |
| 0 | | | | | | | 57.7AU |

| | MDA-MB-231 | | | | |
|---|---|---|---|---|---|
| | 10 | 20 | 50 | 100 | $R^2$ |
| EGF | | | | | 0.91 |
| FGF | | | | | 0.86 |
| HGF | | | | | 0.03 |
| VEGF | | | | | 0.53 |
| PDGF | | | | | 0.06 |
| MIF | | | | | 0.27 |
| MCP1 | | | | | 0.11 |
| RANTES | | | | | 0.00 |
| MIP1a | | | | | 0.00 |
| IFNg | | | | | 0.00 |
| TNFa | | | | | 0.38 |
| TNFb | | | | | 0.93 |
| GMCSF | | | | | 0.60 |
| IL1a | | | | | 0.11 |
| IL1b | | | | | 0.02 |
| IL2 | | | | | 0.39 |
| IL4 | | | | | 0.21 |
| IL5 | | | | | 0.61 |
| IL6 | | | | | 0.99 |
| IL8 | | | | | 0.82 |
| IL10 | | | | | 0.00 |
| IL12 | | | | | 0.30 |
| IL13 | | | | | 0.40 |
| FITC | | | | | 0.24 |
| 0 | | | | | 8.9AU |

The secretome mediated pathway of cell migration is an adaptive process dictated by cell signaling. Understanding this mechanism provides a potential strategy towards decreasing the metastatic capacity of cancer cells [1].

**Highlights of IsoPlexis' Functional Proteomics and Secretomic Signatures in Tumor Cell Migration**

- Functional phenotyping revealed IL-6 and IL-8 were secreted at high concentrations in a specific ratio and density-dependent manner.
- Proteins typically associated with promoting tumor metastasis and progression were not elevated, suggesting that IL-6 and IL-8 are responsible for driving the density-dependent cell migration within 3D matrices.
- The data reveals a possible mechanism for the promotion of tumor cell migration while inferring an approach to reduce metastatic capability of tumor cells.

Jayatilaka H, et al. Synergistic IL-6 and IL-8 Paracrine Signalling Pathway Infers a Strategy to Inhibit Tumour Cell Migration. Nature Communications, 8, 15584, 2017

## Application 2 – Inhibitory Mechanism of Monocytes Toward Immune Suppression in Follicular Lymphoma (FL)

*Functional Phenotyping Identifes Mechanistic Importance of Monocyte Function in Immmune Suppression*

**Products Used**

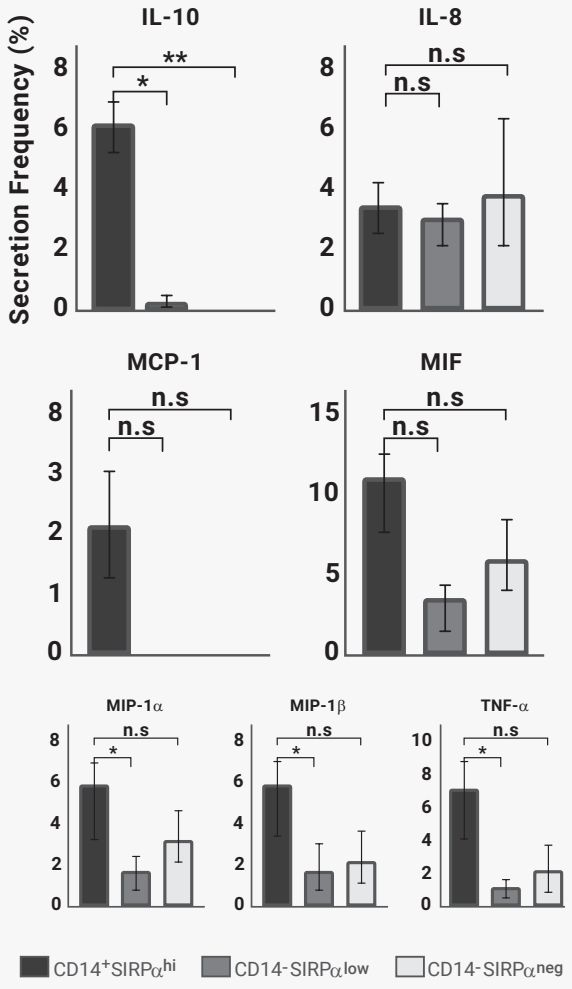
- Single-Cell Secretome (human)
- CodePlex Secretome

### Highlights of Prognostic Response with Single Monocyte Function

- CD14+SIRPα$^{hi}$ (signal regulatory protein-α) monocytes/macrophages were associated with an inferior survival in FL, & increased numbers of the CD14−SIRPα$^{low}$ subset appeared to correlate with a better survival.
- Cellular IL-10 level shows critical mechanistic importance of monocyte function in the interaction with T cell functional response, which correlated with patients who had inferior survival.
- Revealed an inhibitory mechanism of enhanced IL-10 in single-cell polyfunctional CD14+ SIRPα$^{hi}$ subsets reducing T cell proliferation, which provides the functional insights of Mo/MΦs subset for FL patients, which were implicated in inferior survival of patients.

Chen Y-P, et al. SIRPα expression delineates subsets of intratumoral monocyte/macrophages with different functional and prognostic impact in follicular lymphoma. Blood Cancer Journal, 2019

### Cytokine/Chemokine Production by CD14+SIRPα$^{hi}$, CD14−SIRPα$^{low}$, or CD14−SIRPα$^{neg}$ cells




Cytokine/chemokine production by CD14+SIRPα$^{hi}$, CD14−SIRPα$^{low}$, or CD14−SIRPα$^{neg}$ cells determined by the the Single-Cell Innate Immune solution. Results were expressed as the percentage of cells producing cytokines/chemokines. *p < 0.05; **p < 0.01; n.s. no significant difference. N = 3.[2].

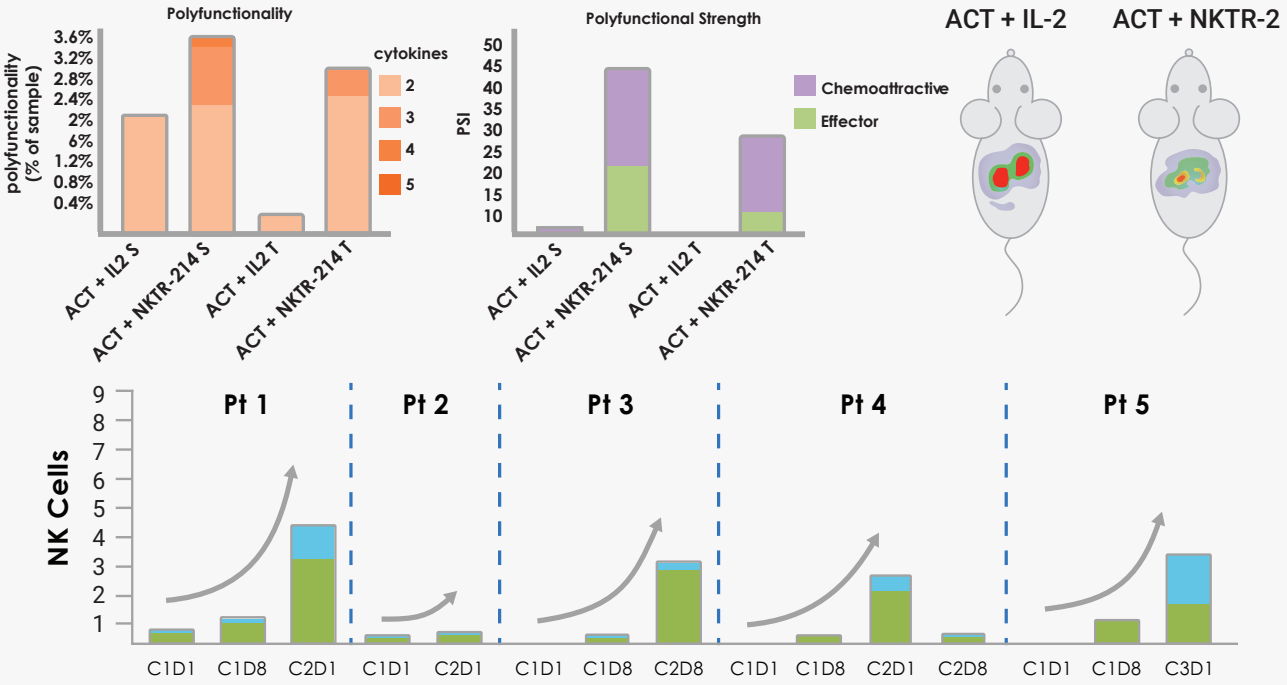
## Application 3 – Mechanisms of Immune Persistence in Solid Tumor Indications

*Functional Phenotyping Identified Biomarkers of Tumor Killing, Expansion, and Persistence*

**Products Used**

Single-Cell Secretome (Mouse)

### Single-Cell Functional Mouse Correlates Drive Pre-Clinical Insights into PEG-IL2 Impact & Superior Mechanism




In solid tumor patients undergoing PEG-IL2 therapy, IsoPlexis' platform revealed upregulation of function in circulating CD4+ and CD8+ T cells and NK cells in the clinical setting in response to this innovative therapy [3].

**Applying Single-Cell Secretome to Cellular Novel Combination Therapies**

- Single-cell functional phenotyping was used to profile translational insights between early stage development of combination PEG-IL2 with ACT therapy, and clinical impact on various immune cell types.
- Systemic polyfunctional mouse immune cell response was seen in response to PEG-IL2, which correlated to the increases of proliferation, homing, and persistence of anti-tumor T cells *in vivo*.
- The mechanism resulted in superior anti-tumor activity in B16-F10 murine melanoma model as well as the promotion of polyfunctional T and NK cells in peripheral blood of patients with melanoma in a phase 1 clinical trial.

Parisi G, et al. Persistence of adoptively transferred T cells with a kinetically engineered IL-2 receptor agonist. Nature Communications, 2020

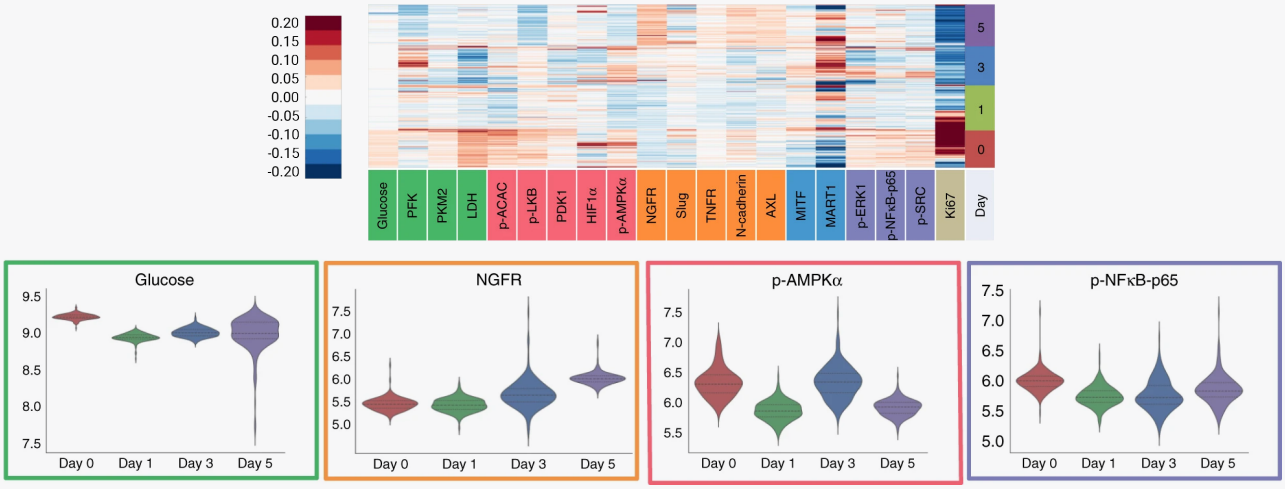
## Application 4 – Resolving Tumor Heterogeneity to Reveal Independent Trajectories of Drug Tolerance

*Multi-Omic Analysis of Signaling, Phenotypic, and Metabolic Regulators to Overcome Drug Resistance in Solid Tumor*

**Products Used**

- Single-Cell Intracellular Proteome
- Single-Cell Metabolome

### Capturing Metabolites, Cytoplasmic Proteins, and Phosphoproteins from Single Cells with IsoPlexis' Proteomic Barcoding Technology




Single-Cell Intracellular Proteome and Single-Cell Metabolome were used to analyze mutant melanoma cancer cells (BRAFV600E M397) to gain further information about the transition from drug responsive to drug tolerant [4].

### Highlights of Multi-Omics Analysis for Drug Resistance in Solid Tumor

- Drug resistance is a major problem in cancer treatment. Although many gene expression studies have tried to elucidate the mechanism of drug resistance in solid tumors, no previous studies have truly mapped the proteomic path(s) to resistance at the single-cell level.
- Using a well-characterized melanoma cell line that is known to rapidly develop drug resistance, multiple impactful analyses revealed changes in functional phenotype over time in individual cells.
- IsoPlexis' Single-Cell Intracellular Proteome/Metabolome technology revealed multiple independent paths that tumor cells take in the development of drug resistance.

**Su Y, et al. Multi-omic single-cell snapshots reveal multiple independent trajectories to drug tolerance in a melanoma cell line. Nature Communications, 2020.**

## Prep, Run, Analyze

### Challenges & Applications

**Application 1:** Understanding Cancer Cell Communication to Infer a Strategy to Inhibit Tumor Cell Metastasis

**Application 2:** Inhibitory Mechanism of Monocytes Toward Immune Suppression in FL

**Application 3:** Mechanisms of Immune Persistence in Solid Tumor Indications

**Application 4:** Resolving Tumor Heterogeneity to Reveal Independent Trajectories of Drug Tolerance

### Solutions

- CodePlex Secretome reveals secretomic signatures in tumor cell migration
- Single-Cell and CodePlex analysis identifes mechanistic importance of monocyte function in immmune suppression
- Single-Cell Secretome identifies biomarkers of tumor killing, expansion, and persistence
- Single Cell Intracellular Proteome and Metabolome reveal multiple independent pathways to drug tolerance in solid tumor

## References


1. Jayatilaka H, Tyle P, Chen J, Kwak M, Ju J, Kim HJ, Lee JSH, Wu PH, Gilkes DM, Fan R, and Wirtz D., Synergistic IL-6 and IL-8 Paracrine Signalling Pathway Infers a Strategy to Inhibit Tumour Cell Migration., Nature Communications, 8, 15584., 2017.
2. Chen Y-P, Kim HJ, Wu H, Price-Troska T, Villasboas JC, Jalali S, Feldman AL, Novak AJ, Yang, Z-Z, and Ansell SM., SIRPα expression delineates subsets of intratumoral monocyte/macrophages with different functional and prognostic impact in follicular lymphoma, Blood Cancer Journal, 2019
3. Parisi G, Saco JD, Salazar FB, Tsoi J, Krystofinski P, Puig-Saus C, Zhang R, Zhou J, Cheung-Lau GC, Garcia AJ, Grasso CS, Tavare R, Hu-Lieskovan S, Mackay S, Zalevsky J, Bernatchez C, Diab A, Wu AM, Comin-Anduix B, Charych D, and Ribas A., Persistence of adoptively transferred T cells with a kinetically engineered IL-2 receptor agonist. Nature Communications, 2020
4. Su Y, Ko ME, Cheng H, Zhu R, Xue M, Wang J, Lee JW, Frankiw L, Xu A, Wong S, Robert L, Takata K, Yuan D, Lu Y, Huang S, Ribas A, Levine R, Nolan GP, Wei W, Plevritis SK, Li G, Baltimore D, and Heath JR., Multi-omic single-cell snapshots reveal multiple independent trajectories to drug tolerance in a melanoma cell line. Nature Communications, 2020