TECHNICAL NOTE

Access the Breadth of Sample Preparation Protocols to Awaken True Functional Immune Biology

Precedent Protocols and Processes ensure that enrichment, stimulation and labeling will result in maximizing the correlative data achieved, while minimizing non-specific impact on the cells themselves.

Highlights:

- Published enrichment, stimulation, and staining protocols throughout immunotherapy types and indications help achieve your goals, in multiple publications and presentations
- Protocols that minimize impacts on the live cells that are analyzed on Bruker's IsoLight and IsoSpark platform
- Guidelines for most commonly used cell types and applications

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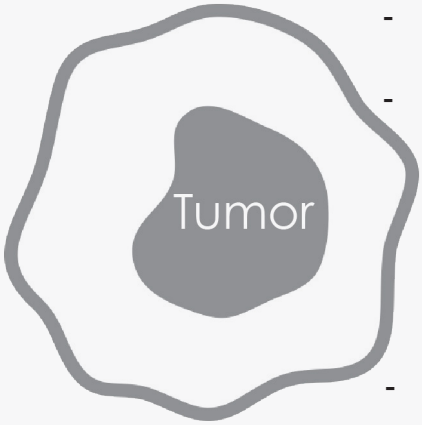
Protocols to engage polyfunctional T cells in checkpoint and combination therapies

PSI correlative biomarkers in a wide body of work

- Checkpoint therapy,
- Combination agonists,
- Double/ triple combination checkpoint
- Immune fitness



- Cellular cancer vaccines,
- Checkpoint therapy
- Combination vaccines
- Bi-specifics
- TCR-T cell Therapies

Proven protocols awaken correlative biology

From accessible blood sample, requiring lower volume of cells

Figure 1 | Our polyfunctional biomarkers reveal the uniquely correlative T-cell specific mechanism in TILS/Bone Marrow microenvironment in a variety of therapeutic areas: checkpoint therapy, combination agonists, Double/ triple combination checkpoint, and Immune fitness. PSI reveals these unique correlates in the blood as well, which presents a more actionable & accessible source of sample in: Cellular cancer vaccines, Checkpoint therapy, Combination vaccines, Bi-specifics, and TCR-T cell Therapies. Our proven Protocols Awaken Correlative Cytokine Biology with stimulation to reveal true functional t cell biology..

Protocol frameworks where Bruker systems achieved published correlates: workflows

Checkpoint & Combination Therapies						
IsoCode Single Cell Protocols	Immune Cell Type	Immunotherapy Type	Indication	Enrichment	Stimulation Type & Time	Polyfunctional Correlate/ Difference
PBMC: T Cell Protocol (H)	Human PBMC[13]	Pre and post cancer vaccine[13]	Cancer: Pancreatic Cancer	Miltenyi Beads	CD3 / CD28: 24 hours	Survival correlate
PBMC: T Cell Protocol (H)	Human PBMC[11]	Pre and post TLR Agonist Therapy[11]	Cancer: Sarcoma	Miltenyi Beads	CD3/CD28: 7 hours	TCR Seq Correlate
PBMC: T Cell Protocol (H)	Humanized Mouse PBMC[21]	Nanoparticle cancer vaccine[13]	Cancer: Solid Tumor	Miltenyi Beads	CD3 / CD28: 24 hours	Mouse In Vivo Correlate
T Cell Protocol (H)	Human Bone Marrow[19]	Combination Checkpoint Therapy	Cancer: Leukemia	Miltenyi Beads	CD3 / CD28: 24 hours	Response Correlates
T Cell Protocol (H)	Human Bone Marrow[19]	Combination Checkpoint Therapy	Cancer: Leukemia	Miltenyi Beads	CD3 / CD28: 24 hours	Newly Diagnosed & Relapse
TILs Protocol (H)	Human TILs[12]	Post Checkpoint Therapy[12]	Cancer: Solid Tumor	Miltenyi Beads	CD3 / CD28: 24 hours	Responder correlate
TILs Protocol (H)	Human TILs[20]	Triple Checkpoint Therapy	Cancer: Ovarian Cancer	Miltenyi Beads	CD3 / CD28: 24 hours	Functionality Correlates
Bispecific Protocol (H)	Human PBMC[18]	Bispecific T cell engager	Cancer: Solid Tumor	Miltenyi Beads	Bispecific T cell engagers: 36 hours	Preclinical Correlate
TILs Protocol (M)	Mouse TILs[16]	Post combination therapy	Cancer: Melanoma	Miltenyi Beads	CD3/CD28: 24 hours	Mouse In vivo response

Figure 2 | The goal for starting with a straightforward protocol that is recommended by Bruker is to ensure success and drive optimization. These protocols have been highly successful in detecting polyfunctional differences in the past (see Technology Note: Validation of Sample Preparation). We recommend not deviating from published protocols, but at the same time simplifying certain aspects that should not impact the success of the study, to ensure success for first time IsoLight users.

Protocols to engage polyfunctional T cells and immune cells in cell therapies

PSI correlative biomarkers in a wide body of work

- CAR T Therapies
- TCR-T Therapies
- NK cell Therapies
- Bispecific Therapies
- CRISPR edited Therapies

- Checkpoint therapy
- Combination vaccines
- Bi-specific CAR T optimization
- TCR-T cell Therapies

Proven protocols awaken correlative biology

Directly from pre-infusion product, or blood, requiring lower volume

Figure 3 | Uncover True functional t cell biology and discover the subsets of cells that make the difference in engineered cell therapies: CAR T , TCR T, NK Cell, Bispecific, and CRISPR edited therapies. PSI reveals these unique correlates in Immun-oncology: Checkpoint therapy, combination vaccines, BiSpecific CAR T Optimization and TCR T cell therapies. Our proven Protocols Awaken Correlative Cytokine Biology with stimulation to reveal true functional t cell biology directly from the preinfusion product or patient blood samples.

Protocol frameworks where Bruker systems achieved published correlates: workflows

Cell Engineering & Therapy						
IsoCode Single Cell Protocols	Immune Cell Type	Immunotherapy Type	Indication	Enrichment	Stimulation Type & Time	Polyfunctional Correlate/ Difference
CAR-T Protocol (H)	Human CD19 CAR-T Cells[2]	CAR-T Therapy	Cancer: Non-hodgkins Lymphoma	Miltenyi Beads	CD19 K562 target cell: 20 hours	Responder Correlate
CAR-T Protocol (H)	Human CD19 CAR-T Cells[6]	CAR-T Therapy	Cancer: B cell ALL	Miltenyi Beads	CD19 Beads: 24 hours	Product Heterogeneity Differences
TCR-T Protocol (H)	Human TCR-Engineered Mart-1 T cells[7]	TCR-T Therapy	Cancer: Melanoma	Tetramer	MART-1 tetramer: 12 hours	Tumor relapse correlate
NK Cell Protocol (H)	Human NK Cells[10]	NK Cell Therapy	Cancer: Leukemia	Miltenyi Beads	IL-15 O/N + IL-12 / IL-18	Mouse tumor regression correlate
Bispecific Protocol (H)	Human Bispecific CAR-T Cells[18]	Bispecific CAR-T Therapy	Cancer: B cell ALL	Miltenyi Beads	CD19/CD22 K562: 20 hours	Process Optimization Characterization
TILs Protocol (M)	Mouse TILs[16]	Combination ACT	Cancer: Melanoma	Miltenyi Beads	CD3/CD28: 24 hours	Mouse In vivo response
CAR-T Protocol (M)	Mouse CD19 CAR-T Cells[17]	CAR-T Therapy	Cancer: B-cell acute lymphoblastic leukemia	Miltenyi Beads	3T3-mCD19 Target cell: 24 hours	Young vs Aging Functional Correlate

Figure 4 | The goal for starting with a straightforward protocol that is recommended by Bruker is to ensure success and drive optimization. These protocols have been highly successful in detecting polyfunctional differences in the past (see Technology Note: Validation of Sample Preparation). We recommend not deviating from published protocols, but at the same time simplifying certain aspects that should not impact the success of the study, to ensure success for first time IsoLight users.

Protocol frameworks where Bruker systems achieved published correlates: workflows

Vaccine						
IsoCode Single Cell Protocols	Immune Cell Type	Therapy Type	Indication	Enrichment	Stimulation Type & Time	Polyfunctional Correlate/ Difference
PBMC: T Cell Protocol (H)	Human PBMC / T cells[13]	Cancer Vaccine	Cancer: Pancreatic Cancer	Miltenyi Beads	CD3/CD28: 24 hours	Survival correlates
PBMC: T Cell Protocol (H)	Humanized Mouse T Cells[21]	Cancer Vaccine	Cancer: Melanoma	Miltenyi Beads	CD3/CD28: 24 hours	In Vivo Response
PBMC: T Cell Protocol (M)	Mouse T cell[22]	Malaria Vaccine	Infectious Disease: Malaria	Miltenyi Beads	CD3/CD28: 24 hours	In Vivo Response
Inflammation & Autoimmunity						
IsoCode Single Cell Protocols	Immune Cell Type	Therapy Type	Indication	Enrichment	Stimulation Type & Time	Polyfunctional Correlate/ Difference
Monocyte Protocol (H)	Human Monocyte in healthy and diseased patients[14]		Autoimmune & CNS: Multiple Sclerosis	Miltenyi Beads	PC3 or LPS: 24 hours	MS correlate
CAR-T Protocol (H)	Human CAR T cell Product[2]	CAR-T Therapy	Immune Related Adverse Events: Neurotoxicity	Miltenyi Beads	CD19-K562: 20 hours	Grade 3+ CRS/ Neurotoxicity
T Cell Protocol (H)	Human T cells[23]		Autoimmune: Systemic Lupus Erythematosus	Flow Cytometry	No Stimulation	Inflammatory state correlate
NK Cell Protocol (H)	Human NK cells, no therapy[8]		Autoimmune: IBD and Crohn's disease	Flow Cytometry	PMA/Ionomycin: 12 hours	Inflammatory state correlate

Figure 5 | The goal for starting with a straightforward protocol that is recommended by Bruker is to ensure success and drive optimization. These protocols have been highly successful in detecting polyfunctional differences in the past (see Technology Note: Validation of Sample Preparation). We recommend not deviating from published protocols, but at the same time simplifying certain aspects that should not impact the success of the study, to ensure success for first time IsoLight users.

Bruker Protocol Frameworks to ProvideUsers with Precedent Workflows

Bruker systems have been used in a variety of research areas and contexts, as seen in Figure 2, 4, 5 to achieve understanding of correlates to in vivo activity in patients and mice. The below figure provides an overview of the variety of research areas, cell types, and indications where Bruker systems have been used to publish data to achieve correlates to response, i.e. patient differences, that explain mechanism in vivo. The Stimulation type and enrichment type can act as a guide, with cited publications and presentations, that users can reference.

See Figure 2, 4, 5 for a variety of contexts in which our protocols for stimulation, enrichment, and labeling were used to achieve correlative outcome.

Conclusion

- Published enrichment, stimulation, and staining protocols throughout immunotherapy types and indications can help provide a framework to achieve goals of obtaining single-cell data, in multiple publications and presentations
- These enrichment and stimulation protocols minimize impacts on the live cells that are analyzed in Bruker's systems
- The type of sample preparation protocols and strategies have helped researchers achieve critical correlates to in vivo data in the past as well
- While every user project is specific in terms of requirements, (e.g., stimulation time), precedent datasets can act as a rubric for choice of protocol.

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