Immunotoxicity Contract Services

Immunotoxicity: A 2-Tier Testing Platform

The lymphopoietic / immune system is often considered separate to the hematopoietic system. In fact they are liked by common primitive stem cell populations present in the adult bone marrow. One of these is the High Proliferative Potential-Stem and Progenitor Cell (HPP-SP), which is situated at the point where lymphopoiesis diverges from hematopoiesis. From this primitive stem cell, the T- and B-lymphocyte progenitor cell populations are formed and from them, the functional immune cells. Immunotoxicity testing can, therefore, be divided into a 2-tier testing platform. The first tier determines if an agent affects the stem and lymphopoietic progenitor cells. The second tier focusses on the functional immune cells. If a response to an agent is observed during the first tier of testing, the effects will also be seen during the second tier of testing.

Tier 1: *In Vitro* Predictive Immunotoxicity Testing

To initially investigate immunotoxicity, it is recommended to assess the effect of drugs or compounds on 3 primary cell populations:

- HPP-SP, primitive lympho-hematopoietic stem cell
- T-CFC, primitive T-lymphocyte progenitor cell
- B-CFC, primitive B-lymphocyte progenitor cell

using the ImmunoGlo™-Tox HT in vitro platform.

Benefits of Using ImmunoGlo™-Tox HT

- Provides predictive information on whether an increase or decrease in immune cell numbers can be expected.
- High throughput (96- or 384-well plate) screening of immunotoxicants.
- Incorporates the most sensitive ATP bioluminescence proliferation/cytotoxicity readout available.
- Fully standardized and validated assay readout allowing results to be compared over time.
- 10-100 more sensitive than WST-1 or CSFE).
- Available for multiple species.
- Rapid turnaround time: usually 4-7 days.
- Designed for multiplexing

Tier 2: *In Vitro* Testing of the Immune System

The second step of in vitro immunotoxicity testing involes specific assay to measure the number and response of T- and B-cell populations and subtypes.

- T-lymphocyte proliferation.
- B-lymphocyte proliferation.
- Mixed lymphocyte proliferation (using ImmunoGlo™-MLC).
- Cytotoxic T-cell (CTL) response.
- NK response.
- Mitogen and co-factor stimulation.
- Dendritic cell maturation and co-stimulation
- Cytokine production and release.
- Immunostaining and flow cytometry of T-cells and subsets.
- Immunostaining of NK cells.
- Immunostaining of B-cell substets.
- Chemokine response
- Phagocytic ability

The above studies are usually performed on human peripheral blood cells. However, other animal species are also available as are the ability to use purified immune cell populations.

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Immunotoxicity Testing Panel and Assay Multiplexing

Assay Name	Assay Type	Pathway	Readout
ImmunoGlo™-Tox HT	Intracellular ATP (iATP)	Stem cell, T- and B- progenitor cell cytotoxicity (Tier 1 or Tier 2)	Bioluminescence
ImmunoGlo™-MLC	iATP	Mixed lymphocyte culture / T- and B-cell proliferation (Tier 2)	Bioluminescence
HALO®-Tox HT	iATP	Lympho-hematopoietic cell populations cytotoxicity (Tier 1 or 2)	Bioluminescence
CAMEO™-4	Clonal, methylcellulose	Lympho-hematopoietic cell populations (Tier 1 or 2)	Differentiation/Colony number (manual)
CAMEO™-96	Clonal, methylcellulose/iATP	Lympho-hematopoietic cell populations (Tier 1 or 2)	Proliferation (Bioluminescence) / Colony number (manual)
FloDiff™	Immune membrane markers (e.g. CD3, 4, 8, 19, 73)	Characterization/ Differentiation (Tier 2)	Fluorescence
Cell cycle	DNA marker	Proliferation (Tier 2)	Fluorescence
Annexin V / PI	Apoptosis/Necrosis	Phosphatidylcholine (Tier 2)	Fluorescence
CaspaseGlo™	Apoptosis	Caspases (Tier 2)	Bioluminescence
GFkine™	Growth factor, cytokine production/release	Multiple (Tier 2)	Multiple
OxyFLOW™	Oxidative DNA damage	Multiple	Fluorescence

Parameters Used to Define the Study

- Number of test compounds.
- Number of reference, positive and/or negative compounds.
- Primary assay(s) to be used and add-on assays to be performed.
- Type and number of cell populations to be tested.
- Type and number of species to be tested.
- Compound dose range.
- Number of compound doses (usually 6-12).
- Type of test compound addition.
- Culture conditions.
- Curve fit analysis, number of EC/IC values (if relevant) and statistics (if applicable).
- GLP/non-GLP/QA audit.

Our Contract Services Workflow

- The CSO of HemoGenix® will advise and consult with our clients regarding the best assays to perform to achieve the goals of the study.
- Prepare quote and revise if required to suit budget.
- Prior to the start of the study, a detailed Study Plan will be prepared by the Study Director for the Study Monitor's approval.
- Sponsor shipment of test compounds.
- Initiate study on arrival of tissues.
- Completion of study usually less than 1 week for HALO® or up to 2 weeks for CAMEO™.
- Phase I report provided in 5-7 business days after study completion.
- Audit of study data and Phase I report.
- Phase II Final Report and study termination.