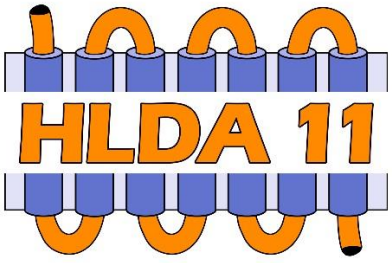


**HLDA11 WORKSHOP PROTOCOL**

HLDA Workshops are *wet* workshops based on an international exchange and blind evaluation of mAbs, submitted by numerous academic laboratories and/or companies

The main goal has consistently been to identify mAbs reacting with a common Ag.

The basic strategy is to assess a given mAb's reactivity with a large number of different cells, followed by statistical analysis of the resulting expression data and further examine of the biochemical nature and molecular mass of the target antigen and the reactivity with transfected cells.

**HLDA11**

**Step 1.** MAbs are submitted by academic groups and/or companies and one panel of mAbs against G protein-coupled receptor. (see attached list of potential new CDs).

Amount of antibody: 500 micrograms (PE-labeled) for FACS analysis; and 100 micrograms (unlabeled) for IP and IHC

Submission dead-line June 2019

**Send us the HLDA11 submission form before sending antibodies (pengel@ub.edu).**

**Step 2.** The organizing laboratory aliquots and distributes the mAbs among the participating laboratories

**Step 3**. Participating laboratories perform reactivity blind studies with the mAbs included in the panel using 12-color flow cytometry.

- Primary blood primary cells (all mayor leukocytes and lymphocyte subsets) two tubes of 8-color flow cytometry. Other cells (platelets, erythrocytes)

  Tube 1. Innate leukocytes (markers: CD16, CD56, CD14, CD11c, HLA-DR; CD123, and CD3, CD19, CD34, DAPI)

  Tube 2. Lymphocyte subsets. (markers: CD45, CD3, CD19, TCRg/d, CD4, CD8, IgM, IgD, CD45RA, CD27, CXCR5, CD25)

A minimum of 12 samples (4 in 4 different labs)

- Collection malignant cells (leukemia's, lymphomas and myeloma cells) to be determined

  A panel of a minimum of 20 leukocyte cell lines and 5 non-hematopoietic cell lines. Suggested list to discuss: T cell lines: Jurkat, HUT78, Molt4; B cell lines: REH1, RAJI, DAUDI, SUDHL10, UPN2, U266, RPMI8226; Myeloid cell lines: HL60, THP-1, KG1, EM2, K562; NK cell lines: YT; Non-hematopoietic: COLO205 (colon), A549 (lung), AN3CA (uterus), HEK (kidney), HELA (adenocarcinoma), MGHU3 (bladder carcinoma), MCF7 (breast)

This approach is only possible as a combined effort by a large group of laboratories

Dead-line December 2019

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**Step 4**. Statistical analysis clustering (code brake)

- Clustering within the panel and with the data obtained by the CDMaps project.

**Step 5.** Biochemical characterization of the target molecules using immunoprecipitation (data are used to further validate the clustering analysis). Only mAbs against potential new CDs.

**Step 6:** Reactivity with transfected cells. The cross-reactivity of the Abs with proteins encoded by a common gene family.

**Step 7:** Reactivity with Malignant cells (leukemias)

A cohort of about 150-200 CLL all well characterized for several surface markers (including expression, activation state of the integrins VLA-4 VLA-3 and VLA-5) and molecular markers (including mutations of TP53, NOTCH1 BIRC3 and SF3B1)

**Step 7**. CD designation. The designation of new CDs requires submission to the workshop of at least two independent mAbs that recognize the same molecule and present an identical pattern of reactivity

**Step 8**. All of the generated data and experiments performed with the submitted mAbs are presented at the HLDA Conference, probably in conjunction with an international Immunology meeting.