

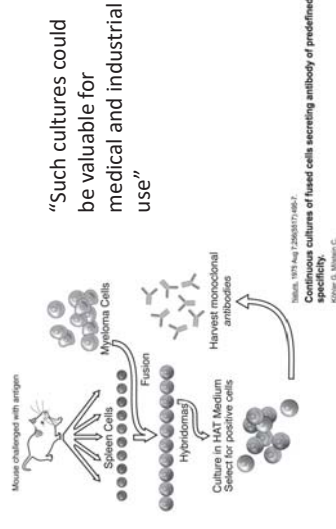
Immunotherapy's Impact on Cell Based Assays

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Immunotherapy

Use of Igs for Immunotherapy

Monoclonal Antibodies:



Brief history:

- * Animal Serum
- * Rh Immune Globulin
- * Monoclonal Antibodies

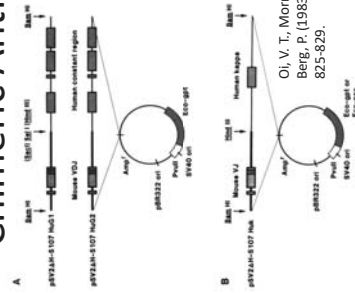
Chimeric Antibodies:

"It also will facilitate development of antibody molecules for immunodiagnosis and immunotherapy."

Human Monoclonal

Production of human hybridomas secreting antibodies to measles virus.
CROOK, C.M., LUMENBACH, A., HALL, W., SIEPKLEWSKI, Z., KOZDROWSKI, H.

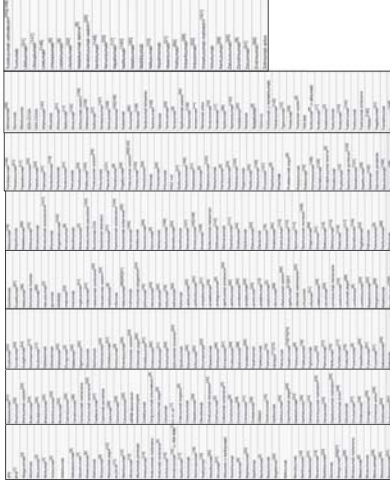
We have hybridized a hypoxanthine phosphoribosyl transferase (Hprt)-deficient human B-cell line derived from a patient suffering from multiple myeloma with peripheral lymphocytes obtained from a patient with subacute sclerosing panencephalitis (SSPE). These hybridomas were found to secrete human IgM specific for measles virus nucleocapsids.



Humanized Antibodies

Transgenic mice:

Human Ig Genes into Mice and Sensitizing the Mouse with Ag.



Lexicon

- -mab = monoclonal antibody
- -tumu- = human
- -tumo- = mouse
- -tuxi- = chimeric
- -tuzu- = humanized

What is available

Source

humanized
chimeric/humanized
human
human
human
humanized
mouse
human
humanized
human

https://en.wikipedia.org/wiki/List_of_therapeutic_monoclonal_antibodies

Enough background, now on to the issues.

Human antibodies interfere with diagnostics in ways that were not necessarily anticipated.

Histocompatibility

Blood Bank

Alloantibody Screening
Compatibility Testing

2 Examples:

- Rituximab α CD20
- Daratumumab α CD38

HLA Laboratory

Routinely perform

Flow Cytometric

Compatibility Assay

3 color flow crossmatch

α CD3 – T cells – HLA Class I

α CD19 – B cells - HLA Class II

AHG – detect bound IgG

to HLA Class I and/or Class II

B cells –

*Reason α CD20 not used.

*CD20 is IgG FcR

*High background if present

*Reduce background using

Pronase to remove CD20

Rituximab (α CD20)

*commonly used in desensitization protocols

Rituximab

Becoming a more commonly used therapeutic for autoimmune disease

Recipient has Rituximab

prophylactically:

*binds to residual CD20

on B cell, it is human/humanized Ig

**AHG detects

False pos or False neg????

Blood Bank Laboratory

Daratumumab

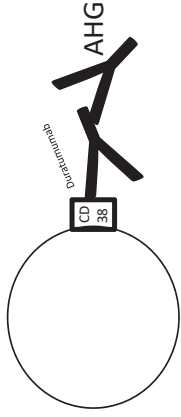
Anti-CD38

“-umu-” so a human Ig

*CD38 expressed on hematopoietic cells

CD38

- Cyclic ADP ribose hydrolase
- Ectoenzyme catalyzes synthesis and hydrolysis of cyclic ADP-ribose
- Regulation of intracellular Ca²⁺
- Receptor mediated adhesion
- Expressed on B, T, NK, plasma cells and red blood cells, among many others

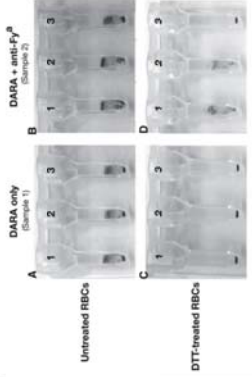


Resolutions:

- * Dithiothreitol (DTT)
- * Trypsin
- * Soluble CD38/anti-DARA

- * RBCs – even see pos DAT and low Hgb
- * Panagglutination – Screens, Panels, XMs

Transfusion, 2016 Sep 7, doi: 10.1111/trf.13789. [Epub ahead of print].
International validation of a dithiothreitol (DTT)-based method to resolve the daratumumab interference with blood compatibility testing.
 Chapoux C¹, Aqaied MQ², Nicholson RT³, AufBuchen JP⁴, Cobio CS⁵, Delaney M⁶, Fung MK⁷, Unger M⁸, Doshi P⁹, Murphy ME¹⁰, Dumont L^{11,12}, Kaufman RM¹³, DARA-DTI Study Group* for the BEST Collaborative.



Issues with using DTT:

Removes Kell antigens

Recommendations:

- * Kell type the recipient
- * Use Kell negative donors



Questions?