General Guidelines for Metal Selection for Antibody Labeling - Panel Development

The CyTOF is currently tuned for a mass window approximately AW 103-203. The high end and the low end of the mass window have lower signal intensities compared to the middle of the mass range. Maximum sensitivity is centered slightly higher than the middle of the range. The lanthanide metals are La139-Yb176, and will vary in signal intensity by no more than a factor of 3-4.

1. Expression level of marker
    - Higher expression - dimmer metal
    - Lower expression - brighter metal
        - Fold-change caveat: if you're looking at only a small fold-change after stim, you may want a brighter metal for that marker to help distinguish the change.

2. Resolution needed for gating
    - Bivariate gating
        - one bright, one dim (e.g., B cell gating using CD20-Dy164 vs CD19-Nd142)
        - two mediums
            -seldom need for two brights

3. Expression type
    - Bimodal expression - yes/no - eg CD27
        - dimmer metal (easy to distinguish positives from negatives)
    - Spectrum of expression- "smear" - eg CD45RA, CCR7 somewhat
        - brighter metal may be needed to allow finer distinction in bivariate second dimension

4. If you need to redesign a panel, shifting metals up to two masses up or down will generally not impact signal/resolution.

5. Tm169 is the brightest metal for the CyTOF tuned to the AW 103-203 mass window for MAXPAR reagents/lanthanides.
    * If you don't see signal with Tm169, you're not going to see signal.

6. Metal impurities/oxides and effect on signal
    - The metals that DVS Sciences sells as part of their MAXPAR kits are of high purity (98-99% in most cases). This means that "compensation" analogous to fluorescent antibodies isn't typically needed, as most of the signal will be mass "M", with little to no signal at "M+1" or another contaminating mass (La139 is often a contaminant in salts that's difficult to separate out using chemical methods).

- Mass M, and Mass M+1 - some isotopes are less pure than others. For example, Nd145 from DVS, and definitely Gd155, Gd157, Dy161, Dy163, and Yb173 (DVS does not sell due to purity concerns; we obtained from Trace Sciences) have contaminating metals, usually other isotopes of the same lanthanide. The largest contaminant is usually the M+1 isotope.
    - Therefore, be careful using the less-pure isotopes.
        - Consider using them for "dump" channels.
        - Put a lower-abundance marker at a less-pure "M" so that the spillover (usually up to 0.5-1% of "M" signal) is reduced.
        - If "M" is a less pure isotope and is labeling a high-abundance marker, don't put a low-abundance marker at the M+1 position. Aim for at least medium-abundance so that positive and negative populations can still be clearly resolved if there is isotopic "spillover."
        - If you must, try to use exclusive/orthogonal combinations of markers. For instance, put an exclusively-T cell marker at M+1 after an exclusively-B cell marker labeled with a less-pure isotope M.
- Mass M, and M+16 - most lanthanides exhibit some degree of oxidation in the argon plasma. Proper tuning of the Current and Make-up Gas each day can help minimize this (oxide <3% of maximum signal), but some degree will always be present. Technically oxide formation decreases signal at M, while increasing signal at M+16. You are more likely to notice a percent or few increase in oxide "background" at M+16 than you will the percent or few decrease in signal at M.
    - Some lanthanides have more oxidation than others.
        - La139 is the worst (oxide mass 155)
        - Nd (oxide mass 158-166) and Gd (oxide mass 172-176) have notable levels as well
        - Eu (oxide mass 167, 169) has comparatively low oxide levels
    - Try to put a lower-abundance marker at more-easily oxidized "M" so that M+16 spillover (again, usually up to 0.5-1% of "M" signal) is minimized.

<u>Important QC/QA points:</u>

1. Know when marker expected to be expressed.
    - Cell types
        - Monocyte, NK cell, T cell, B cell, etc
        - Location - eg, peripheral circulation vs bone marrow
    - Effects of stimulation or other variable - eg, test on stimulated cells if not predicted to be expressed on unstimulated cells.
    - Effects of sample processing

- some markers (e.g., CD62L) lost on cryopreserved PBMCs. Often come back after resting.
- some cell types lost after processing (e.g., granulocytes and dendritic cells after Ficoll gradient of whole blood for PBMCs)
- staining after fixation and/or permeabilization can destroy epitopes (CD16 after PFA), or, conversely, cause false positives (large increase in anti-CD56 mAb binding after PFA)

2. Positive *and* negative controls
   - Different cell types within same sample: using B cells as negative control for T cell markers, etc.
     -Caveat: Beware of limitations of this, where a marker may be expressed by more than one type of cell
   - If doing secondary staining (2° labeled streptavidin against 1° biotinylated mAb, 2° labeled anti-fluorophore antibody against 1° fluorophore-mAb, etc)
     - Negative control (2° stain in absence of 1° mAb)
     - Validate 2° stain against a good, stable marker (biotinylated or fluorophore-labeled CD3, CD4, CD45RA, etc) prior to use with new marker; helps ensure any problem would be with the new 1° mAb, not 2° stain.
   - Cell lines: proteinatlas.org - immunohistochemistry data for ~4300 proteins on 47 cell lines
     - Caveat: the antigen expression on a cell line may be higher or lower than seen on primary samples
     - Caveat: samples were fixed, paraffin-embedded, deparaffinized with xylene, rehydrated with ethanol, boiled in antigen-retrieval solution, stained, then read by a computer.
       - In other words, the staining may not exactly match what you get by flow

3. Validate against more than 1 donor. Some donors have anomalous expression.
   -False negative/Undercounting
     - Example: some donors have low or no CD33 expression (thereby think that the CD33 antibody doesn't work).
   -False positive/Overcounting
     - Example: others have anomalously high numbers of CD8+ CD28+ cells (thereby missing that the CD28 mAb had false positive/overcounting/T cell-specific background problem).