Using Innovative Cytometry Solutions To Support The 3 Pillars of Drug Discovery

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Introduction

Generating data to support the 3 pillars of drug discovery greatly increases the prospects of a potential therapeutic medicine making it to market. Our biomarker group has employed both traditional and innovative ways of measuring endpoints. Within this, we focus on the 3 pillars for monoclonal antibodies against cell surface targets. Herein we describe the technologies we use, the types of data generated, and how this information helps refine the models used for dose prediction, thereby improving clinical outcomes.

Pillar I

To prove exposure at the site of action for peripheral targets both flow cytometry (binding assays), and imaging cytometry can be used. The ImageStream™X MKII is used within our group to generate internalisation data for potential cell surface target therapeutics. We do this both pre-clinically to inform doses/differentiate molecules, and to complement Phase I studies in PK data need further interpretation.

METHOD: PBMC from healthy volunteers are incubated with two different fluorescently labelled potential therapeutics at 4°C, before washing away unbound antibody. Cells are then incubated at 37°C for varying periods before fixation and immunofluorescence is performed. Use of fluorescently labelled competitive or non-competitive mAbs after fixation, gives us the ability to discern free receptor can be recycled back to the cell surface. Representative images of the ImageStream™X are shown below. The IDEAS software was used to determine fluorescence measurements and internalisation scores.

RESULTS:

Figure 1: Risk management matrix for use in clinical development based on the Three Pillars

Figure 2: Fluorescence intensity (+/- STD 4 donors) of FITC-anti-target A mAb (green) in the membrane of CD8+ T cells decreases only due to internalisation of the mAb (arrows) which begins within 1 hour. In parallel, binding of a competitive anti-target A mAb increases, demonstrating availability of free target A.

Figure 3: Representative images of CD4+ T-cells from 1 PBMC donor. Red = non-competitive anti-target B mAb, yellow = anti-target A (green) + anti-target B (red), green = anti-target A + anti-target B (green), blue = anti-target A (green) + anti-target B (red) + anti-target C (blue). At 4°C, both the therapeutic mAbs and the non-competitive mAbs are found on the cell membrane. Incubation occurred over 1 time course (B: 5 hours, C: 29 hours) with a concurrent decrease in the amount of free target on the cell membrane as shown in a reduction in non-competitive mAb binding (red). For comparison D: shows expression of free target A on the cell membrane. In this case cells have been treated with a much lower concentration of anti-target B mAb than used in A/B/C but also treated for 29 hours after wash off.

Figure 4: Representative ZellScanner™ images (anti-CD20 = purple, anti-target X = yellow) taken from the cynomolgus macaque study [2], and incubated for 24 hours after washing. The lymphocytes (a target tissue) from all animals were taken at the CRW and sent to GSK Stevenage for analysis by flow cytometry. The aspirates were loaded onto ZellSafe™ chips and stained with a fluorescently labelled anti-CD20 antibody (to define the B cell population) and fluorescently labelled anti-target X (the same mAb that was dosed) to look at free receptor on the surface of these B cells. Blood samples were taken in parallel for flow cytometric analysis.

Figure 5: Receptor occupancy on blood from the same animals as the traditional flow cytometry shows a dose dependency and suggests 1 mg/kg is a saturating dose.

Figure 6: Basic workflow used for the SMART tube system

METHOD: SMART tubes were pre-loaded with lyophilized cytokine (lyospheres) at a specified concentration. Blood from healthy volunteers was drawn into solution from heparin vacutainers and then aliquotted into blank or lyosphere loaded SMART tubes. Tubes were placed into the base station and a specified period of incubation at 37°C was begun using a pre-loaded program. At the end of the incubation period, the base station then breaks the ampoule of preservation solution within the tube, rotates it to mix and incubates for a further 10 minutes. Samples are then ready for freezing/analysis by flow to measure pSTAT5/C phosphorylation.

RESULTS:

Figure 7: Bivariate dot plot showing pSTAT5/C expression taken from FlowJo analysis of blood incubated in blank and lyosphere loaded SMART tubes. A: FSC/SSC properties are different as expected but immunophenotyping (CD) and detection of pSTAT5/C (D) is clear and well defined. Q2 carried out a full validation of this assay.

Pillar II

To demonstrate target engagement (TE) whole blood receptor occupancy (RO) flow assays can be used if the target is expressed by peripheral leukocytes. However, in situations where it may be crucial to show TE in the tissue if sample is scarce, we have used chip cytometry (Zellscanner™ Ono, ZellKraftWerk), a system based on iterative imaging cytometry.

SUMMARY: Traditional flow cytometry performed alongside innovative chip cytometry data confirm that saturation is reached at the doses tested, and that the anti-target X mAb can reach the target tissue and bind the receptor within 24 hours of dosing. These data are also used by the modellers to improve clinical dose predictions.

Pillar III

Pharmacological activity can be determined using routine flow cytometry assays such as intracellular cytokine staining and phospho-flow. Often these responses are unstable and/or transient. This can be managed in preclinical studies but can create logistical challenges when the assay moves into the clinic. To counter this we have used pre-loaded tubes allowing for measuring phosphorylated proteins in the clinical environment. Proof-of-concept work was carried out at GSK and then transferred to Q solutions for validation for use in a clinical trial.

Conclusion

As demonstrated above, Cytometry is well placed to deliver data supporting all 3 pillars and is therefore a critical platform for the advancement of medicines. Continual innovation in the cytometry field will allow scientists to generate higher content data faster and more reliably, thereby allowing modellers to refine dose and regimen predictions in advance of clinical protocol planning. These innovations will also help overcome the logistical problems encountered in large scale, multi-centre clinical trials, resulting in robust data more reflective of what is happening at the bed-side.

Other future solutions?

Automated bedside flow cytometry – the AccelJet from LeukoDx. All reagents are held in a flat plate format; temperature stable cartridge, meaning minimal intervention is required. The instrument has been used in multiple clinical studies, currently but could prove useful for multi-centre clinical trials.

All human biological samples used in the work described above were sourced ethical and as such research was in accord with the terms of the informed consents under an IRB/EC approved protocol

All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures Act 1986) and the GSK policy on Care, welfare and treatment of animals.

References


Source: smarttubeinc.com

Source: leukodx.com

Source: ZellKraftWerk.com