Multiplex Assays with Coregistration Enables Multiple Biomarker Analyses Across Tissue Sections

A case study in developing a multiple biomarker strategy to empower drug development decisions

Introduction

Summit Therapeutics, a life sciences company developing a therapy for Duchenne muscular dystrophy (DMD), was faced with a drug development challenge. They needed to accurately differentiate between on-going muscle regeneration and therapeutic impact of its development compound, ezutromid, in dystrophic muscle. Summit experts in DMD and utrophin modulation teamed up with Flagship experts in automated tissue biomarker qualification to deliver a solution for the evaluation of muscle biopsies in Summit's clinical trials. In this case, multiplex assay development quantifying multiple biomarkers through coregistration, is able to clearly identify the impact of the therapeutic candidate versus regeneration.

Client Problem

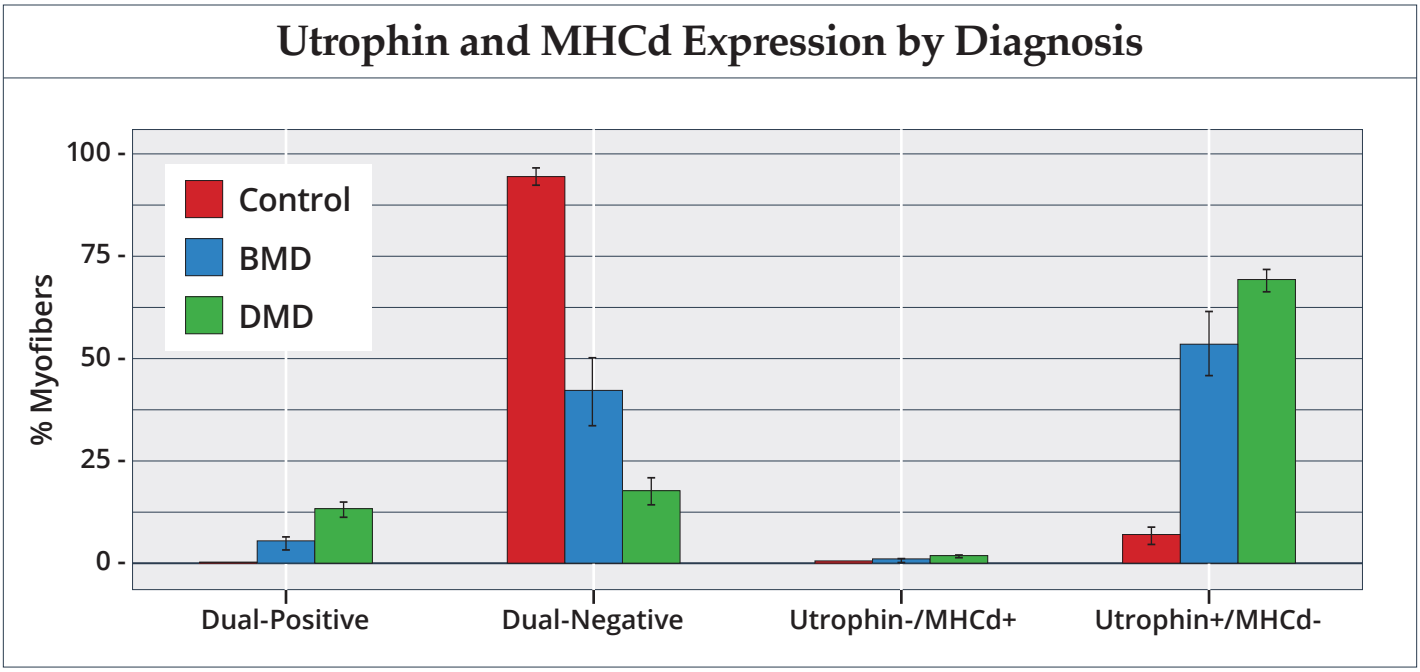
In DMD, loss of the structural protein dystrophin from muscle fiber membranes results in fragile fibers that are easily damaged during normal muscle function. Over time, this fragility results in progressive muscle degeneration. One potential treatment for DMD that Summit is developing modulates utrophin, a developmentally regulated structural protein that has the potential to functionally substitute for dystrophin. Utrophin is also transiently expressed during fiber regeneration, which complicates measurements of therapeutic efficacy. The challenge is how to distinguish persistent utrophin expression due to therapeutic effect from natural utrophin expression caused by on-going regeneration. In order to differentiate utrophin expression from therapeutic effect versus on-going muscle regeneration, we designed a strategy to evaluate three biomarkers: a fiber identifying marker (laminin alpha2), therapeutic target (utrophin) and an independent marker of regeneration (developmental myosin heavy chain) in the same fiber. However, multiplex assay development presents increasingly challenging hurdles as the number of biomarkers increases. A few of the key technical challenges involve antibody quality, cross-reactivity, and differentiating signal.

Technological Solution

Flagship's MuscleMatch™ algorithm co-registers images of serial tissue sections stained with duplex assays, allowing quantification of multiple biomarkers in the *same fiber*. Using this method, fibers are identified and matched across sections using the laminin alpha2 stain present in each duplex. Once the fibers are matched, we then quantify expression of the second biomarker in each duplex assay – utrophin in one, MHCd in the other. The result is a measurement of both key biomarkers in the same fiber, allowing fibers expressing utrophin likely due to therapeutic effect (utrophin+; MHCd-) to be distinguished from fibers expressing utrophin due to on-going regeneration (utrophin+; MHCd+)(Figure 1).

Case study by Flagship BIOSCIENCES

Figure 1: Utrophin and MHCd Expression by Diagnosis



Examination of utrophin and MHCd subpopulations reveals fewer dual-negative fibers and more utrophin+/MHCd– fibers in DMD samples than in BMD and CTRL samples. Differences in dual-positive and utrophin–/MHCd+ fibers are much smaller between diagnoses. Error bars represent the standard error of the mean (SEM). Samples were obtained from the Wellstone Muscular Dystrophy Cooperative Research Center for tool development purposes.

Value to Client

This approach provided client value by helping to clarify a complex situation so that assessment of drug effect can be performed with confidence. Coregistration is empowering therapeutic efficacy evaluations where a biological feedback loop makes interpretation of protein expression anything but straight-forward. But this technique comes with other advantages as well. Coregistration provides flexibility for dynamic drug development programs. With Flagship's Computational Tissue Analysis (cTA®) platform, duplex assays are developed and analytically validated on an as-needed basis and select assays are coregistered to provide exactly the information needed to drive confident go/no-go decisions in clinical trials.