

Leveraging Quantitative Imaging Cytometry to Measure the Pharmacodynamic Impact of Drugs in Development

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Dr. Juan has led the imaging cytometry group at Amgen Inc. since 2005. In close collaboration with a cross-functional team of scientists, she is using state-of-the-art microscopy to develop and validate pharmacodynamic biomarkers to enable clinical decisions. Dr. Juan received her doctorate from the University of Valencia, Spain, where her research focused on the development of flow cytometric methods to assess metabolic toxicity in whole cells. As a Postdoctoral Fellow in Dr. Darzynkiewicz's laboratory at New York Medical College and later as an Assistant Professor there, she was involved in research projects primarily focused on analysis of cell cycle regulation and apoptosis, work that continued at Memorial Sloan Kettering Cancer Center, centering on translational cancer studies.

Abstract

Mast Cells (MCs) have important functional roles in leukocyte recruitment, pain, and wound healing, and increased tissue-resident MC function has been associated with several fibrotic diseases. Consequently, the study of MCs *in situ* can be a direct approach to studying the pharmacodynamic impact of MC-directed therapeutics in tissues

Skin is a readily accessible site for biopsy sampling, and the dermis contains numerous mast cells (MCs). Published studies have shown that cells in the bed of skin wounds produce increased levels of Stem Cell Factor, which contributes to trafficking and accumulation of tryptase-positive MCs near the healing injury (1-3). Experiments in mice and non-human primates confirm that measuring MC accumulation near healing wounds is a feasible approach to assess MC-directed therapies *in vivo*. For this purpose, an automated laser scanning cytometry (LSC) assay was used to characterize the kinetics of MC accumulation in healing skin wounds and to study the effect of inhibiting CD117 (cKit) signaling. The number of tryptase-positive MCs approximately doubled 14 days after cutaneous injury in nonhuman primates. Treatment of animals with anti-CD117 or imatinib mesylate (Gleevec[®]) reduced MC accumulation at the edge of healing wounds in mice and nonhuman primates, respectively (4). In addition, a cutaneous wound model of MC infiltration has been reported in humans with ~5-fold increase from baseline in MC number (5). In this model, MCs were initially depleted from the incisional wound area. Over a period of two weeks, MCs repopulated at the edges of the healing tissue.

In translating this automated LSC-MC assay to become a biomarker for human studies, we performed an unblinded exploratory study to evaluate cutaneous wound models of MC infiltration in healthy volunteers. From this study, we concluded that MCs recruit to healing wounds with delayed but measurable kinetics in humans. This finding is distinct from the published report by Trautmann *et al.*, and we propose that second-intention wounding with a more protracted healing time than previously described is necessary to observe MC accumulation. Additionally, because MCs are highly variable between subjects, they should be evaluated with an automated assay in longitudinal studies.

This assay is being implemented in a Phase I clinical study.

1. Galli *et al.* J Clin Invest 1993, 91: 148-152
2. Huttunen *et al.* Arch Derm Res 2002, 294: 324-330
3. Meininger *et al.* Blood 1992, 79: 958-963
4. Zoog *et al.* Cytometry 2009, 75A: 189-198
5. Trautmann *et al.* J Path 2000, 190: 100-106