is the pathologic formation of endochondral bone in soft tissue commonly occurring after severe trauma. HO is a SMAD-signaling dependent process typically occurring in response to an acute inflammatory insult. Traditionally targeted therapeutics for treatment of HO have focused on mesenchymal cell progenitors. However, macrophages which are integral orchestrators of the initial inflammatory response and are primary agents of cytokine secretion in wound regeneration, have received little attention. Here we identify a role of macrophage production of TGFβ in HO and demonstrate a role for macrophage targeted therapies in the treatment and prevention of this debilitating condition.

METHODS: Wild type and macrophage-specific TGFβ knockout mice (LysM-cre/Tgfb<sup>fl/fl</sup>) mice underwent hindlimb Achilles’ tendon transection and dorsal 30% TBSA burn. Mice were euthanized at 2, 5, and 12days, and 3 and 9 weeks after injury. A concurrent cohort of wild type animals was treated with i.v. trabectedin (0.15 mg/kg), a macrophage-depleting agent, administered twice or vehicle control to assess the effect on HO. The tenotomy site of wild-type mice harvested at 2-, 5-, and 12-days were digested to a single-cell suspension, stained with antibodies against macrophage markers (Ly6G, Ly6C, F4/80, CD11b) and TGF-β for flow cytometric analysis. MicroCT images were obtained at 9 weeks after injury among wild type and TGFβ knockout mice, and among trabectedin and control-treated mice. In vitro and in vivo studies were performed to validate a novel PLGA microparticle system with preferential uptake by macrophages.

RESULTS: Flow cytometry of tissue from wild type mice demonstrated enrichment of TGFβ in F4/80+ macrophages at all time-points with macrophages present as the dominant inflammatory population by 12-days post-injury (76%; p<0.05). LysM-cre/TGFβ mice similarly demonstrated a significant reduction in tHO volume vs. wild type controls (7.49 v. 1.25 mm<sup>3</sup>, p=.007). Macrophage depletion via trabectedin led to a 12.9-fold decrease in tHO volume when compared with control-treated mice (p<0.008). Histologic analysis at 3-weeks confirmed diminished cartilage presence in both mutant and trabectedin-treated mice when compared with respective controls. Finally, the PLGA microparticle demonstrated preferential uptake by macrophages both in vitro and in the wound site in vivo.

CONCLUSION: We demonstrate that TGFβ is present in macrophages at the tenotomy site and that these macrophages comprise an increasingly large portion of the inflammatory infiltrate through 12-days post-injury. Global inhibition via trabectedin therapy is effective in preventing tHO. Importantly, genetic loss of TGFβ among macrophages was sufficient to significantly reduce HO. Our findings are strengthened by the development of a novel microparticle which exhibits preferential uptake by macrophages for drug delivery.

3
Identification of Fibroblast Subtypes during Hypertrophic Scar Development

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PURPOSE: The complex biological mechanisms occurring in hypertrophic scarring are still barely understood. However, the identification of (myo)-fibroblasts provides us with clues in determining the pathophysiology of fibrotic changes. Here, we focus on analyzing dermal fibroblast subtypes in murine dorsal skin following hypertrophic scarring in an in vivo model. We aim to understand the role of fibroblast subpopulations in the skin during healing and to determine their involvement in fibrotic scar development.

METHODS: Hypertrophic scars (HTS) were induced by placing a biomechanical loading device onto the dorsum of C57/BL6 mice. On day 4 after causing an incisional wound, which was reapproximated immediately with 6-0 nylon sutures, the mechanical device was placed and tension was applied twice per day for 11 days. On day 11 all devices were removed. The scars of 10 HTS-mice were examined on day 11 and day 18 post-incision. Unwounded skin of C57/BL6-mice served as controls. Hypertrophic scars were identified via staining. The skin/tissue was digested and single cells were subjected to fluorescence-activated cell sorting to isolate cells of interest. Cells were negatively sorted for CD45, CD31 and EpCAM (CD326) and positively sorted for CD34, CD26 and CD55 to isolate fibroblasts and functional fibroblast subpopulations.

RESULTS: FACS analysis revealed a change in the subpopulations of fibroblasts following mechanical stretching of the skin. Specifically, we observed decrease of the stemness marker CD34 in hypertrophic scars compared to...
unwounded skin. These changes were observed on day 11 as well as day 18 post-incision. CD34 is frequently used as a stemness marker and a marker of adipose-derived stem cells (ASCs). Hypertrophic scars were verified via staining (100% sensitivity).

CONCLUSIONS: For the first time, our results reveal a decrease of the stemness marker CD34 in murine skin cells in hypertrophic scars over time compared to unwounded skin. We are currently conducting single cell transcriptional analyses and functional studies on these fibroblasts to understand the heterogeneity of fibroblasts during hypertrophic scarring and the specific role of CD34+ fibroblasts in skin fibrosis. Our findings have promising therapeutic implications for the treatment of skin fibrosis.

4

Morbidity and Quality of Life Outcomes of Breast Reconstruction for Unilateral Mastectomy vs. Additional Contralateral Prophylactic Mastectomy: a Cohort Study of 211 Breast Reconstruction Patients

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PURPOSE: The rates of contralateral prophylactic mastectomy at the time of therapeutic mastectomy for unilateral breast cancer have more than tripled in the past decade, reaching 12.7% of cases. This is despite the lack of evidence for survival benefit associated with these procedures. Indeed, there is a lack of published data on postoperative outcomes for cases of contralateral prophylactic mastectomy followed by bilateral breast reconstruction (CBR) compared to unilateral mastectomy and breast reconstruction (UR). We performed the current study to investigate potential differences in morbidity and patient-reported quality of life (QoL) outcomes between these two groups.

METHODS: Using our IRB-approved, prospectively collected breast reconstruction patient registry, we queried pre- and post-operative data from patients who underwent CBR or UR at our institution. Data included patient demographics, comorbidities, surgical history, cancer treatment, pre-operative and 12-month post-final reconstruction Breast-Q© scores, breast reconstruction treatment, and post-operative complications. We used simple and multiple linear regression to compare morbidity and QoL changes between the study groups (CBR vs. UR). Satisfaction with abdomen domain was not included in the analyses due to the heterogeneity of reconstruction types, however, type of reconstruction was adjusted for in the adjusted analysis.

RESULTS: Between 2010 and 2015, 211 patients underwent CBR (n=86, 40.8%) or UR (n=125, 59.2%). While the unadjusted surgical morbidity was significantly higher for the BR group at 60 days post-tissue expander placement (p < 0.001), it was not significantly different between groups immediately before final reconstruction, at 60 days post-final reconstruction, or at 1 year post-final reconstruction. After adjusting for age, BMI, type of reconstruction, timing of reconstruction, chemotherapy, radiotherapy, and previous breast surgery, CBR patients did not have a statistically significant difference in pre- to post-reconstruction changes of QoL when compared to UR in the domains of Satisfaction with Breast (p=0.62), Psychosocial Well-being (p=0.71), Sexual Well-being (p=0.85), and Chest Physical Well Being (p=0.09).

CONCLUSION: Our findings suggest that performing a contralateral prophylactic mastectomy at the time of therapeutic mastectomy and bilateral breast reconstruction for unilateral breast cancer is not associated with higher QoL compared to unilateral mastectomy and breast reconstruction. While there was no increased morbidity at 1 year post-final breast reconstruction, there was a higher rate of short-term (60-day) complications for staged breast reconstruction following tissue expander placement for the CBR group. These results would help in counseling patients interested in undergoing contralateral prophylactic mastectomy and bilateral breast reconstruction for unilateral breast cancer.

5

Analysis of Survival and Recurrences for Patients with Breast Cancer Receiving Mastectomy with or without Breast Reconstruction