Background Low-dose radiation therapy (LD-RT) in combination with immune checkpoint inhibition (ICI) and/or cell therapy has emerged as an effective mediator to restore immune response in solid tumors. However, clinically available biomarkers still fail to effectively predict response and optimize therapy choices. Emerging evidence indicates that nanomechanical alterations of the tumor microenvironment are viable predictors of aggressiveness. We hypothesized that the benefit of LD-RT lies not only in immune activating, but also stromal and tumor nanomechanical remodulation by LD-RT. In this work we have investigated the nanomechanical signature of tumor response to LD-RT combined with immunotherapy, to study nanomechanical drivers of immune infiltration, and how they can be used to optimize cancer diagnosis and orientate therapy choice.

Methods For the ICI study, 344SQ lung adenocarcinoma tumors resistant to anti-PD1 treatment were established in 129Sv/Ev mice. Mice were treated with a combination of anti-PD1 and anti-CTLA4 antibodies, with and without LD-RT pre-treatment. For the cell therapy study NSG mice were implanted with gastric carcinoma cells. Both low dose and high dose RT were used in combination with cell therapy. Survival and tumor growth were monitored for the various groups. Tumor biopsies were collected at early time points and at the end of each experiment. All tissues were examined via the ARTIDIS platform, to extract a multiparametric nanomechanical signature. The ARTIDIS technology used, combines atomic force microscopy with artificial intelligence proprietary algorithms to provide accurate and reliable characterization of the nanomechanical properties of cancer tissue. We also performed histopathology, multiplex immunofluorescence and Nanostring analyses to characterize stroma remodulation and immune infiltration.

Results In both studies we have identified a clear signature of radiation – induced enhanced response to immunotherapy, in the nanomechanical parameter space. In the radiation dataset we achieved almost perfect separation of responders vs non responders, with 90% sensitivity, 99.1% specificity and 96% AUC. In the cell therapy dataset, we can clearly detect the signature of T cell infiltration post radiation, with 92% sensitivity, 100% specificity and 95% accuracy, and this is valid across different doses of T cell infused and for both low and high dose radiation.

Conclusions In these studies, we demonstrated for the first time a clear nanomechanical signature of LD-RT-mediated response to both immune checkpoint inhibitors and cell therapy in two different mouse models. Our findings open the way to using ARTIDIS nanomechanical signature as a clinically translatable predictor of response to combination radiation and immunotherapies.