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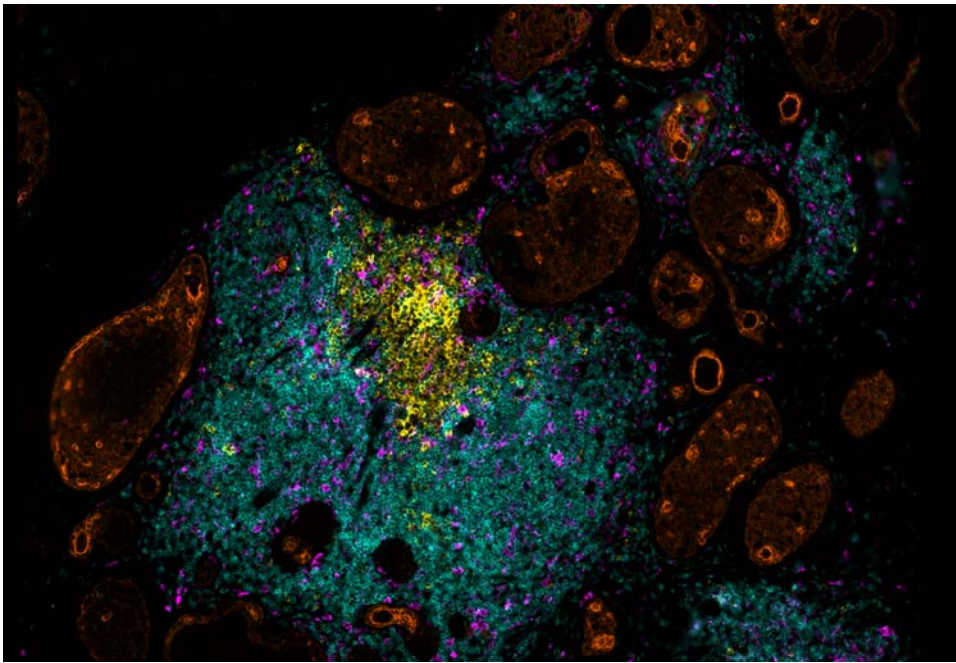
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The Iafrate laboratory has focused efforts on developing highly complex molecular analyses of tumor genetics using novel technologies. We have a strong interest in the clinical implementation of genetic screening technologies that can help direct targeted therapies, focusing on lung, breast and brain tumors. Our recent contributions in the treatment of a subset of non-small cell lung carcinoma (NSCLC) with rearrangements of the ALK tyrosine kinase, rearrangements of the ROS1 tyrosine kinase and MET exon 14 skipping with a small molecule kinase inhibitor (crizotinib), underscore the promise of personalized cancer care (1, 2). We currently are focusing on detecting tumor DNA in blood samples (“liquid biopsies”) to allow for efficient and convenient tracking of cancer progression. In addition we are developing new techniques to allow for early detection of cancers by detecting tumor-specific DNA in circulation.

We have developed and deployed next generation sequencing to detect chromosomal rearrangements in tumor tissue, with on-going studies that assess the relative sensitivity in much larger clinical cohorts. The method we have developed, termed “anchored multiplex PCR” or AMP, is an efficient target enrichment technology, allowing for 100s of targets to be simultaneously analyzed from small tissue samples (3). We have used AMP to screen thousands of tumor samples, and have uncovered numerous novel driver fusion genes. Our lab is now focused on modeling novel fusions in vitro and developing therapeutic approaches to screening these fusions. We have also initiated studies of tumor heterogeneity; these efforts focus on gene amplification of receptor tyrosine kinases in glioblastoma (4). This work has revealed a new subclass of brain tumors with mosaic gene amplification of up to three kinases in distinct but intermingled cell populations within the same tumor, forming a mosaic pattern. We found that each subpopulation was actively proliferating and contributing to tumor growth. Detailed genetic analysis found that different subpopulations within a particular tumor

shared other gene mutations, indicating that they had originated from the same precursor cells. Mapping the location of different subpopulations in the brain of a glioblastoma patient suggested that each subpopulation may serve a different function in the growth and spread of the tumor. Our lab has developed novel highly-multiplexed FISH technology to address how many genes show copy number heterogeneity, and to study the spatial distribution of such populations (5), see image. We are exploring the therapeutic implications of such driver gene heterogeneity in cell line model systems of glioblastoma using genome-wide CRISPR knock out screens.

More recently we have adapted the AMP sequencing technology in other areas, including (1) mapping off-target rates for CRISPR-CAS genome editing; (2) sequencing and mapping the distribution of IgH and TCR rearrangements in tumor samples; and (3) ultra-high sensitive mutation calling in circulating tumor cells and cell free plasma samples. Using AMP we have developed tissue-specific cell-free DNA (cfDNA) panels to examine the most important cancer genes in common tumors, including lung,



Multiplex Immunofluorescence to detect changes in the immune landscape in head and neck tumors.

melanoma, breast and colon cancer. Such panels are allowing us to track, with a simple blood draw, the tumor burden in patients. We are able to use cfDNA analysis in patients with metastatic cancer to see if they are responding to therapy, and also can track the development of resistance mutations. This allows a real-time dynamic optimization of therapy. Most recently we have developed a methylation-based sequencing assay to allow efficient analysis of tumor-specific methylation patterns in cfDNA samples. We hope that such an approach can be a lot more sensitive in the detection of small amounts of circulating tumor DNA, allowing potential early detection of tumors before they are clinically symptomatic. In addition, the methylation patterns are actually specific to the type of tumor the DNA is derived from, potentially allowing us to determine the actual anatomic site of origin.

The lab has developed multiplex immunofluorescence panels to study the spatial biology of tumor types including ovarian cancer (looking at homologous recombination repair proteins) and head and neck cancer (looking at immune infiltrates).

Using the Lunaphore platform, the lab can simultaneously examine >15 markers at true single cell resolution. We have developed computation pipelines to analyze these complex datasets.

Selected Publications:

Garcia-Beltran WF, Iab EC, Astudillo MG, Yang D, Miller TE, Feldman J, Hauser BM, Caradonna TM, Clayton KL, Nitido AD, Murali MR, Alter G, Charles RC, Dighe A, Branda JA, Lennerz JK, Lingwood D, Schmidt AG, **Iafrate AJ**, Balazs AB. Covid-19-neutralizing antibodies predict disease severity and survival. *Cell*. 2021; 21;184(2):476-488.

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Zheng Z, Liebers M, Zhelyazkova B, Cao Y, Panditi D, Chen J, Robinson HE, Chmielecki J, Pao W, Engelman JA, **Iafrate AJ***, Le LP*. Anchored multiplex PCR for targeted next-generation sequencing. *Nat Medicine*. 2014; Nov. 10.

Shaw AT, Ou SH, Bang YJ, Camidge DR, Solomon B, Salgia R, Riely GJ, Varella-Garcia M, Shapiro GI, Costa DB, Doebele RC, Le LP, Zheng Z, Tan W, Stephenson P, Shreeve SM, Tye LM, Christensen JG, Wilner K, Clark JW, **Iafrate AJ**. Crizotinib in ROS1-Rearranged Non-Small Cell Lung Cancer. *N Engl J Med*. 2014; Sept. 27.

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