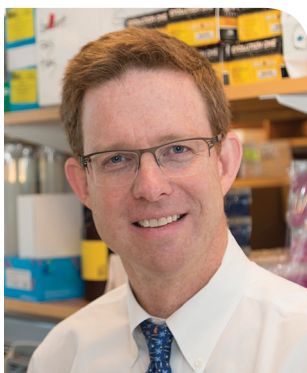


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The Graubert laboratory focuses on the molecular basis of human blood cancers, including acute myeloid leukemia and myelodysplastic syndromes. The laboratory utilizes a variety of genomic platforms to interrogate primary samples from patients with myeloid malignancies to identify inherited and somatic mutations that drive these diseases. The goal of these studies is to gain insight into the biological basis of myeloid leukemias, and to improve strategies for diagnosis, risk stratification, and targeted therapy.

Clonal heterogeneity of myelodysplastic syndromes

Myelodysplastic syndromes are the most common form of acquired bone marrow failure in adults. Despite the ineffective hematopoiesis that is characteristic of this disease in its early stages, we found through whole genome sequencing that nearly all cells in the bone marrow of these patients are clonally derived (see Figure). When patients evolve to acute myeloid leukemia (which occurs in approximately one third of cases), new subclonal populations emerge that are derived from the original (“founding”) clone. These findings raise the possibility that the prognostic value of recurrent mutations in myelodysplastic syndrome and the efficacy of therapies that target these mutations may depend not only on the presence or absence of these mutations, but also on their position within the clonal hierarchy of this disease.

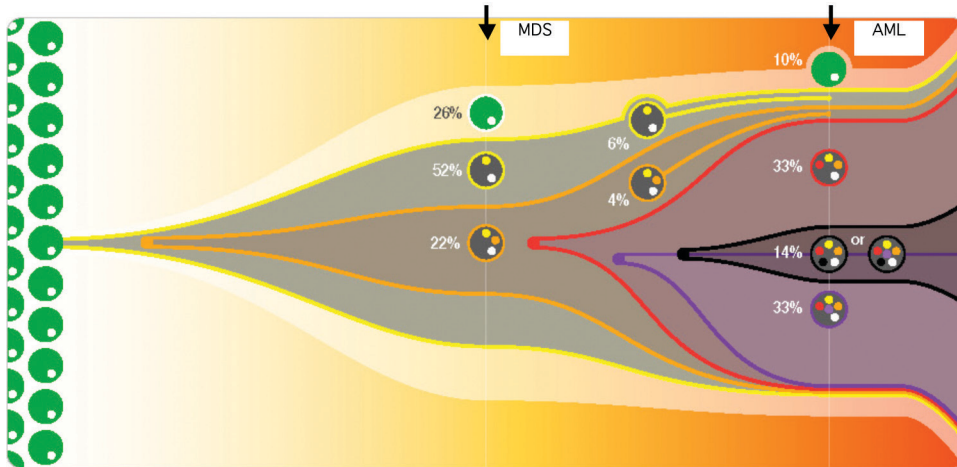
RNA splicing defects at the root of myelodysplastic syndromes

We and several other groups discovered recurrent somatic mutations in genes encoding core components of the RNA splicing complex (the “spliceosome”) in patients with myelodysplastic syndrome. Mutations in this pathway tend to be mutually exclusive, suggesting that more than one splicing gene mutation in a cell provides

no additional selective advantage, or is deleterious to the clone. We have focused on *U2AF1* which encodes a component of the U2 snRNP that binds to the AG dinucleotide at the 3’ intronic splice acceptor site. Mutations in *U2AF1* arise early in the pathogenesis of myelodysplastic syndromes (in the founding clone) and affect almost exclusively two codons in predicted zinc finger domains. We have shown that the most common mutation (S34F) has gain-of-function activity in splicing assays. Current work in the Graubert laboratory is focused on comprehensive analysis of the impact of *U2AF1* mutations on splicing, the functional consequences of these mutations for blood cell development, and vulnerabilities created by splicing gene mutations that provide opportunities for novel therapies.

Inherited predisposition to myelodysplastic syndrome/acute myeloid leukemia

Acute myeloid leukemia and myelodysplastic syndromes are usually sporadic, late-onset cancers, but in rare instances (<1%) these diseases aggregate in families. In these families, predisposition to acute myeloid leukemia/myelodysplastic syndrome may be a consequence of an inherited bone marrow failure syndrome, but in other cases these are highly penetrant, autosomal dominant, Mendelian disorders. Three



Clonal evolution from myelodysplastic syndrome (MDS) to acute myeloid leukemia (AML). Whole genome sequencing at the time of MDS diagnosis (left arrow) in a representative patient identified a founding clone comprising ~52% of the bone marrow cellularity and a subclone derived from the founding clone in ~22% of cells. When this patient progressed to AML (right arrow), the original clones were still present and had spawned three new subclones that were dominant in the bone marrow at this time point.

genes (*RUNX1*, *GATA2*, *CEBPA*) explain fewer than half of these Mendelian cases. The genetic basis in the majority of families is not yet known. Furthermore, the latency and incomplete penetrance of acute myeloid leukemia/ myelodysplastic syndrome in mutation carriers suggest that acquisition of cooperating somatic mutations is required for malignant transformation. We have accumulated a large panel of samples from affected and unaffected members of these families. Ongoing studies in the Graubert laboratory are focused on identification of novel germline variants in families that lack known predisposing factors, and characterization of the landscape of cooperating somatic mutations that arise in these cases. This information is important for genetic counseling in these families, for selection of optimal bone marrow transplant donors, and to increase our understanding of the biological basis of acute myeloid leukemia and myelodysplastic syndromes.

Selected Publications:

- Nguyen HD, Zou L, and Graubert TA. Targeting R-loop Associated ATR Response in Myelodysplastic Syndrome. *Oncotarget*. 2019 Apr 5; 10(27):2581-2582.
- Nguyen HD, Leong WY, Li W, Reddy PNG, Sullivan JD, Walter MJ, Zou L, Graubert TA. Spliceosome Mutations Induce R loop-Associated Sensitivity to ATR Inhibition in Myelodysplastic Syndrome. *Cancer Research*. 2018 Jul 27.
- Brunner AM, Graubert TA. Genomics in childhood acute myeloid leukemia comes of age. *Nature Medicine*. 2018 Jan 9;24(1):7-9.
- Saez B, Walter MJ, Graubert TA. Splicing factor gene mutations in hematologic malignancies. *Blood*. 2017 Mar 9;129(10):1260-1269.
- Nguyen HD, Yadav T, Giri S, Saez B, Graubert TA, Zou L. Functions of Replication Protein A as a Sensor of R Loops and a Regulator of RNaseH1. *Molecular Cell*. 2017 Mar 2, 65(5): 832-847.
- Shirai CL, White BS, Tripathi M, Tapia R, Ley JN, Ndonwi M, Kim S, Shao J, Carver A, Saez B, Fulton RS, Fronick C, O'Laughlin M, Lagisetti C, Webb TR, Graubert TA, Walter MJ. Mutant U2AF1-Expressing Cells Are Sensitive to Pharmacological Modulation of the Spliceosome. *Nat. Communications*. 2017 Jan 9, 8:14060.