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IRB Proposal: CRC Rotation
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1. Study Purpose and Rationale

Treatment of childhood leukemia has drastically improved over the last 60 years. In 1957, children in the United States with a diagnosis of any type of leukemia had a three year survival rate of only 1.7%.[i] By 2000-2005, the five year survival rate of childhood acute lymphoblastic leukemia (ALL) in Children's Oncology Group (COG) studies had climbed over 90%.[ii] Nevertheless, in children ages 1 to 14, malignant neoplasms constitute the second most common cause of death behind unintentional injury.[iii] In children ages 0 to 19 in 2009, leukemia had the highest incidence of any cancer (4.3 cases per 100,000) and the highest death rate (.7 deaths per 100,000 children).[iv] Due to the high incidence of childhood ALL, its contribution to childhood mortality remains significant despite therapeutic advances that have rendered a good prognosis for many patients. Study of the group of children with poor prognoses is thus imperative.

The improvements in survival have been predominantly driven by preventing relapse, but once relapse occurs prognosis remains poor. The most recent COG data showed that about 20% of children relapse, and of those that do the survival rate is dismal: 15 – 50% 5-year survival, depending on how long after initial diagnosis that relapses occurred.[v] Unfortunately, even though prevention of relapse and 5-year survival improved during the last 15 years in COG trials, survival after relapse remained unchanged. Patients that progress to relapse represent a significant portion of the overall mortality associated with ALL, and as such represents a group that deserves further investigation.

While the mechanisms associated with relapsed ALL remain elusive, advances in genomics offer promising opportunities for better identification of relapsed disease and those patients likely to relapse. The goal of induction therapy in ALL is to induce complete remission, which in the latest Dana Farber Cancer Institute (DFCI) trial was achieved >95% of the time.[vi] Yet, despite complete remission being attained >95% of the time, 15% of the patients relapsed. How can this be reconciled? Vast improvements have been made in the ability to detect minimal residual disease in the bone marrow at the end of induction therapy, i.e., detecting when complete remission has not occurred. In fact, this used to be discerned by morphological detection, and it was found that there needed to be >5% blasts in the bone marrow in order to detect relapsed disease.[vii] This meant that a patient could be deemed to have attained complete remission despite a high leukemic burden at the end of induction therapy, and would be cohorted into the same complete remission group as patients who truly had an absence of leukemic cells. However, there have been advances in detecting small leukemic burdens. One method is quantitative polymerase chain reaction (Q-PCR), which is used to amplify nucleotides in T-cell receptor and immunoglobulin gene rearrangements in order to demonstrate the presence of a clonal population of cells.[viii] The other predominant method is flow cytometry, which identifies antigenic markers commonly found on the surface of leukemic cells.[ix] Both of these methods can detect leukemic cells on the order of 1 in 10,000 to 1 in 100,000 cells, or around 0.01 to 0.001% of the cell population.[x] While Q-PCR and flow cytometry have

vastly improved the ability to detect relapsed disease at the end of induction therapy, they are each associated with certain inadequacies. First of all, deep sequencing techniques offer the possibility of being able to detect clonal populations at lower levels than either of these techniques. Q-PCR is a slow, expensive process requiring patient specific primers. Flow cytometry is faster, but highly subjective and less sensitive.

A future horizon in the detection of currently undetectable minimal residual disease is deep-sequencing. Deep-sequencing is a method of DNA sequencing whereby each nucleotide receives many more reads than in traditional sequencing, with the goal of being able to identify small clonal populations or genes that might portend future relapse. Researchers have already determined that deep-sequencing of end-of-induction bone marrow samples can identify clonality of the immunoglobulin heavy chain (IGH) receptor on the order of 1 in 10^6 cells, or .0001%, in instances where flow cytometry failed to show minimal residual disease.[xi] One prominent study found a gene enriched at relapse called NT5C2, which confers resistance to the chemotherapeutic agents used in maintenance ALL therapy (purine analogs). They were then able to find, through deep-sequencing techniques, presence of the gene of interest in a small clone of cells at diagnosis.[xii] These studies demonstrate the potential of deep-sequencing technology to not only detect minimal residual disease at sensitivities never before attainable, but also to detect somatic mutations present in small numbers of cells that will drive future relapse.

We hypothesize that patients who relapsed after completion of therapy have residual disease detectable in the end of therapy bone marrow test through deep sequencing technologies and that the residual clone was present since time of diagnosis. Furthermore, we hypothesize that the level of residual disease predicts the time to relapse. Deep sequencing analysis for IGH rearrangement of serial bone marrow samples obtained from 29 patients at same time points throughout therapy and at relapse, as proposed in this pilot study, will provide preliminary data to support our hypothesis.

2. Study Design

Dr. Maria-Luisa Sulis has banked diagnosis, end of induction (remission) and end of therapy samples from 29 patients with pre-B-cell ALL, of whom 5 relapsed following completion of therapy and have available relapse sample. These patients were treated with the same chemotherapy regimen on the Dana Farber Cancer Institute Pediatric ALL protocols. all patients had been consented for banking of peripheral blood and bone marrow samples for research purposes. Deep sequencing for clonal IGH rearrangement will be performed by Adaptive Biotech from diagnostic, remission, end of therapy and relapse samples to determine whether presence of detectable disease at the end of therapy is associated with recurrence of leukemia and if the relapsed clone can be tracked back to the time of diagnosis. If our pilot study shows that patients who relapse following completion of therapy have detectable minimal residual disease in the bone marrow performed at end of therapy, the cohort of patients will be expanded to include samples from patients enrolled in the same study through the Dana Farber Consortium.

3. Study Procedure

This study is a translational investigation involving previously banked samples.

4. Study Drugs or Devices

Patients received chemotherapy in accordance with DFCI protocols.

5. Study Instruments (e.g., Questionnaires, Interview Outlines, Focus Group Guides)

Not applicable.

6. Study Subjects

Subjects are 29 patients previously enrolled on DFCI protocols for pre-B cell ALL. These represent the patients whose samples Dr. Maria Luisa-Sulis has been able to collect over the last several years.
Inclusion criteria: patients with pre-B-cell ALL on DFCI protocols with adequate diagnosis, end of induction, and relapse samples.
Exclusion criteria: patients with inadequate samples.
Vulnerable populations: none

7. Recruitment

Patients have previously been recruited on DFCI protocols.

8. Informed Consent Process

Patients were previously consented on DFCI protocols and agreed to have their samples sent for research.

9. Confidentiality of Study Data

Patient samples will be deidentified apart from demographic information.

10. Privacy Protections

Patient information is no longer being collected. All samples will be deidentified during the analysis process.

11. Potential Risks

There are no potential risks apart from a minimal risk of confidentiality breach.

12. Data and Safety Monitoring

All laboratory work will be done in accordance with university rules.

13. Potential Benefits

Insights into utilization of technology to better detect relapse disease. Improved understanding of relapsed disease.

14. Alternatives

None.

15. Research at External Sites

Samples will be deidentified and sent to Adaptive Biotech for high-throughput sequencing of the immunoglobulin domains

16. Columbia as Lead Institution

Columbia is the lead institution of this pilot study.

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[iv] http://www.cdc.gov/cancer/npcr/pdf/USCS_FactSheet.pdf. Accessed 8/27/13.

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