Fight CRC Early Age Onset Colorectal Cancer Workgroup Meeting
Participant and research group summaries

Group 1 Members: Heather Hampel, Dennis Ahnen, Jose Perrea Garcia, Mingyang Song, Phillip Buckhaults and Jessica Martin

As with all the tables, Group 1 discussed; 1) the use of antibiotics in childhood and their effects on the microbiome as a risk factor for early-age onset colorectal cancer (EAO CRC); and 2) the effects of sedentary lifestyle, poor diet (less fresh food and more manufactured food), and other dietary exposures as a risk factor for EAO CRC. However, Group 1 also spent a lot of time discussing the use of somatic genetic profiling to determine the causes of EAO CRC. It is now known that the mutation profiles of tumors create "signatures" that are specific to the underlying cause of the cancer and can be used to predict prognosis[1,2]. For example, there are signatures for tobacco-associated[3], UV-associated[4], and viral (HPV)-associated[5] cancers. It is possible if the microbiome is involved in the increased prevalence of early-onset colorectal cancer, this may create a signature because they alkylate adenine. Obesity might have a signature involving methylation. One study of normal colon tissue has identified eleven ubiquitous signatures which were found in >85% of colon crypts[6]. Interestingly, this study found two mutational signatures in the normal colon that appear to have occurred during childhood. The SBSA signature (characterized predominantly by T>C mutations at ATA, ATT, and TTT and T>G mutations at TTT) appears to be active usually before 10 years of age. The initiating event for this relatively frequent mutational process is unknown, but the results suggest an extrinsic, locally acting and patchily distributed mutagenic insult occurring during childhood. An SBSB signature characterized by C>T substitutions at ACA, T>A at CTN, and T>G at GTG appeared also to be active in the first decade of life. It would be important to determine if these signatures are enriched in early-onset colorectal cancers.

This method for determining the cause of the increased incidence of EOCRC would be to let the tumors tell us their cause based on their mutation signature. This may be superior to "guessing" what is causing the increase based on dietary and lifestyle changes that occurred beginning with the 1960s birth cohort. As we see it, these would be the hypotheses:

- Hypothesis number 1: EAO CRC patients have the exact same mutation risks, and the same profile of mutation signatures as do late onset CRC patients, they are just the unlucky members of the tail of a normal distribution.
- Hypothesis number 2: EAO CRC patients are special; either genetically (doubtful, because an increase in allele frequency and resulting phenotype would not happen over a time span of just 10-20 years) or they encountered some etiological factor that caused a burst in mutation activity at some point in their life.

To answer this question, we propose sequencing tumors from EAO CRC patients, from late onset CRC (LOCRC) patients, and potentially from normal colonic mucosa of controls. It is likely that cohorts exist with tumor DNA or even DNA sequencing results from the EAO CRC and LOCRC cases. Controls may be more difficult to obtain but data is available from previously published studies as described above.

Group 2 Members: Caitlin Murphy, Swati Patel, Luis Diaz, Richard Hayes, Anil Wali, Karen Wehling

Informed by evidence supporting a birth cohort effect[7,8], Group 2 discussed risk factors in early life and opportunities to identify windows of growth and development that confer the greatest risk of early-age onset CRC (EAO CRC). The group prioritized studying: 1) diet and obesity in childhood; and 2) medications in childhood, as risk factors for EAO CRC, similar to the major overarching themes identified across all groups. As a first step, we discussed several ecological studies that can be conducted to correlate incidence trends with these risk factors. Possibilities include: 1) examining incidence rates by birth cohort in other Western and Asian countries; 2) characterizing policy changes in food supply or fortification (e.g., fortification of grain products with folate was authorized in 1996); and 3) describing temporal trends in the approval of antibiotics for children, as well as patterns of use among children. Group 2 also discussed the possibility of linking existing cohort studies, including many of the studies described above, with cancer registry data to study the relationship of diet (often measured via self-report) with EAO CRC. However, we acknowledged that differences in how diet is measured (e.g., assessed during childhood vs. last year) across these studies may make it difficult to isolate the effect of diet during childhood. The group discussed considering alternative time periods, such as adolescence and young adulthood, that may be easier for study participants to recall.

To understand the effect of medications in childhood and risk of early-onset CRC, Group 2 favored using government data from countries with a national health service (e.g., Denmark) that can be linked across multiple registries. These data contain all medications prescribed since birth or immigration into the country and have been used to study medication-related risks for several cancer types.[9,10,11] Using medication fills from a subsample of patients with sufficient enrollment (e.g., from early childhood through mid-adulthood) in an integrated health system would also accomplish this goal. For example, Geisinger Health System has a non-transient, stable patient population, low rates of migration in and out of the area, several life-long residents, and many multi-generation families.[12] These features make it an ideal setting to study risk of early-onset CRC across the entire life course. As an alternative, Group 2 discussed using an existing cohort study of children (e.g., Health Improvement Network)[13] that has routinely collected health information from study participants since a young age. Although these studies of children measure risk factors during relevant time periods, many are limited to children born in the early 1990s and require additional time to mature and yield a sufficient number of cases.

Group 3 Members: Paul Limburg, Rebecca Siegel, Joshua Demb, Andrea Cercek, Jeff Lee, Betsy Risendal Curt Pesmen

At outset, Group 3 members endorsed the hypothesis that environmental exposures are contributing, at least in part, to recently observed increases in EAO CRC incidence and mortality. First, the group agreed that putatively causal environmental exposure(s) must be commonly encountered and widely pervasive to affect similarly unfavorable trends in geographically disparate populations. Applying this contextual framing, multiple broad exposure categories were reviewed, including energy balance, dietary factors, physical activity, microbial agents, air quality, tobacco, alcohol, occupation, and medications. Second, differential disease patterns by race/ethnicity were examined, with the consensus opinion that potential etiologic associations should be comprehensively evaluated across and within race/ethnicity-defined population subgroups. Third, accepting the assumption that an extended period (10+ years) is likely required for colonocytes to progress from normal to malignant, even in the setting of

EAO CRC, the group recommended focusing on exposure history in a target window spanning from childhood to early adulthood. Fourth, limitations were acknowledged referent to existing EAO CRC molecular data. Further efforts to advance current understanding of gene-environment and epigenetic-environment interactions, and how these interactions may be contributing to anatomic site-related differences in EAO compared to older age onset CRC (i.e., proportion of proximal colon, distal colon, and rectal tumors) were strongly supported.

Putting the above elements together, the EAO CRC risk factor combinations of primary interest for the Group 3 were: 1) dietary factors in adolescence/early adulthood; and 2) antibiotic use in adolescence/early adulthood. Many existing resources were identified that could be utilized to extend the current evidence base in these areas, including the National Health and Nutrition Examination Survey (NHANES) Longitudinal Study, Cancer Family Registry, Cancer Prevention Study-3, and National Longitudinal Survey of Youth. The Nurses' Health Study could also be of value, although the baseline age for enrolled participants was at or above the preferred exposure history target window.

Opportunities to generate new data were also explored, including re-activation of longitudinal follow up from the National Children's Health Study and/or childhood obesity clinical trials, leveraging large health system databases (i.e., Kaiser Permanente), or designing community-based case-control studies. Initiation of a prospective cohort study was also discussed, but was deemed untenable given the number of subjects and duration of follow-up required.

Group 4 Members: Jan Lowery, Ann Zauber, Hisham Hussan, Chris Lieu, Yin Cao, Violet Kuchar

Evidence linking increased exercise and fiber-rich diet to reduced colorectal cancer risk has been classified as "convincing" by the World Cancer Research Fund/American Institute for Cancer Research (AICR).[14] In contrast, obesity is one of the strongest risk factors for colorectal cancer.[15] This is likely due to the chronic low-grade inflammation and unfavorable hormonal as well as metabolic profiles seen in obesity.[16,17,18,19,20] Accumulating evidence also links the microbiome to an increased risk of CRC, however more research is warranted to establish firm causative links between the microbiome and CRC risk, progression and prognosis.[21, 22,23]

Group 4 primarily discussed risk factors that may impact EAO CRC such as early changes or prolonged exposure to the following: 1) the colonic microbiome; 2) obesity, specifically visceral obesity; 3) lower physical activity; and 4) low fiber diet consumption and/or increased intake of select dietary items known to adversely affect the risk of CRC.

Ideally, future studies would address 1) the impact of early life and early adulthood prolonged exposure to obesity, decreased exercise, and certain dietary patterns on risk of EAO CRC; 2) earlier alterations in microbiome in EAO CRC patients or young patients with precancerous polyps, do these changes in the microbiome increase risk for CRC independent of other factors when compared to persons without CRC; and 3) has exposure to these factors, overweight, weight-change and dietary factors changed significantly over the past 3-4 decades?

Data sources to support these studies must have the ability to assess microbiome, either directly via tumor and normal tissue at a minimum at the time of diagnosis but ideally, at multiple time points (prior to diagnosis), or indirectly via proxy measures. Sources must also have information on an ample number of subjects with and without EAO CRC in addition to patients with precancerous polyps, weight and diet in childhood/adolescence and updated up to time of diagnosis, comorbidities, and family history in

order to stratify by familial risk or exclude high risk persons due to suspected genetic susceptibility. Few databases will have all the information needed, so it was discussed that an important component to the source selected is ability to re-contact participants.
Major take home message from Group 4: the microbiome plays a critical role in EAO CRC, but there is currently very little evidence to support the biologic plausibility for this hypothesis. Future studies will need to address this gap in order to fully elucidate the role of the microbiome in the pathogenesis of EAOCRC.

Group 5 Members: Steve Waring, Rick Boland, Caleb Levell, Patrick Blatchford, Jordan Karlitz, Claire Palles

Determining the best approaches to reducing the incidence of early- age onset CRC is hampered by gaps in our knowledge regarding the impact of cost, potential harm, efficiency, and cancer location (colon, rectum, both) to inform when and who to screen. This is a unique opportunity to leverage growing interest, capability, and available resources supporting precision 'omics' to accelerate our knowledge towards developing precision screening efforts.
Areas of highest interest include harvesting low-hanging fruit not already picked: 1) identify large cohort studies (regardless of outcome studied) with deep genotype (or tissue for genetic assays) and phenotype and robust follow-up that could be accessed (or if still ongoing, amenable to protocol modifications) to examine:

a) temporal BMI/obesity trends over time
b) nutritional/dietary changes over time impacting the microbiome
c) inflammatory markers
d) somatic mutations
e) emphasis on early childhood/adolescent exposures and 2) need sufficient power to detect relevant effect sizes to begin to inform/transform practice or at least generate significant signals for further study

The no time like the present reality.

Considering that cobbling together existing data and ongoing study potential is still likely to fall short of expectations and may not be sufficiently timely, it is imperative that a large multi-site study be designed and implemented in order to acquire robust genetic, molecular, environmental, and other non-genetic factors and account for racial disparities and differences in risk factors owing to colon vs rectal cancer. This will be the most justifiable approach, albeit an expensive one, if we are to make sufficient progress towards reducing the increasing incidence of and mortality due to EAO CRC. Rigorous research on a large representative cohort that takes advantage of rapidly advancing science and technology would be expected to yield more precise prevention strategies by allowing more accurate risk stratification of individuals most likely to benefit from early screening that could inform health care guidelines and policies that are cost-effective and sustainable.

Online Group 6 Members: Christine Molmenti, Phil Daschner, Roberto Flores, Holli Loomans

The influence of early dietary factors/dietary patterns and the anatomical presentation of the cancer (e.g. colon versus rectum) were consistently mentioned as priority areas for further investigation in EAO CRC. The majority of participants also suggested that the microbiome represents a foundational risk factor which is impacted by diet and other lifestyle patterns, such as increasing sedentary behavior and alcohol consumption in the development of EAO CRC. However, understanding the true effect of each of these risk factors is difficult, as one risk factor (e.g. microbiome) may be directly or indirectly affected by another (e.g. diet) and several modifiable lifestyle factors are likely inextricably linked. Additionally, an individual's underlying genetics may contribute to the magnitude of effect of a given exposure or exposures on the development of EAO CRC. The discussed risk factors illustrate the potential interconnectedness and complexity of EAO CRC etiology. Therefore, Group 6 ultimately decided that evaluating the microbiome first would provide a solid foundation for investigating the other notable risk factors and would provide insight into the role of additional risk factors, such as diet and lifestyle, predominantly investigating these risk factors among the 18-30-year-old age group.

Prospectively obtaining biospecimens, specifically blood, stool, and tumor tissue, and epidemiological data would provide for a thorough investigation the discussed risk factors. Of all currently available studies, there are several that partially fulfill the criteria. For example, the GECCO (Genetic and Epidemiology of Colorectal Cancer Consortium) cohort has conducted a Genome-Wide Association study (GWAS) in coordination with the collection of clinical, epidemiologic, and outcome data to perform risk modeling. The Centers for Disease Control Comparative Effectiveness Research Data Collection Enhancement Project includes information from electronic health records and data linkages to assess clinicopathologic and outcome data. The American Cancer Society's Cancer Prevention Study (CPS-3) is a prospective cohort of 304,000 individuals age 30 to 65 with no history of cancer with the intention of following participants long-term, collecting health and lifestyle data. The above listed studies are incredibly valuable resources, however to meaningfully investigate the etiology of EAO CRC, biospecimens need to be collected alongside epidemiological data (including clinical and demographic data) outlined in the studies above. Participants also discussed the following studies however, further investigation is required to understand if biospecimens and epidemiologic data are available for analysis: NIH All of Us Study, the Growing Up Together Study, and the Diet and Cancer Pooling Project.

In considering potential study design alternatives, there was consensus that designing a large prospective cohort study of young individuals to collect and biobank blood, urine and stool prior to a cancer diagnosis, would provide invaluable insight into pre-initiation exposures, while also collecting tumor tissue, blood, urine and stool at diagnosis. In addition, these data could be further analyzed for the development of risk predication models for risk assessment/risk stratification and in order to determine appropriateness of early screening in this population. Because the etiology of sporadic EAO CRC remains unclear, designing a prospective study to appropriately answer this question should be a top research priority.

Dr. Ann Zauber: Colorectal Cancer Screening Guidelines

Fight CRC deliberately allotted time for Dr. Anne Zauber, to explore research questions since the change in American Cancer Screening guideline in May of 2018 to begin screening for average risk at 45 years (vs 50), and the impact on future guideline updates, specifically: What empirical data does The United States Preventive Task Force need to consider a younger age to begin screening for CRC?
Dr. Zauber provided the following summary, as guideline committees are committed to evidence-based recommendations that balance the benefits and the risks of screening. Microsimulation modeling is also used to provide potential risk and benefit from different strategies. Empirical data is the first consideration in developing population guidelines. The Guideline committees prefer randomized clinical trials demonstrating efficacy. However, they recognize that a randomized clinical trial of age to begin at 45 years versus 50 years of age would not be feasible in the US, due to the large number of people required to assess CRC mortality as an endpoint, and due to the strong potential of contamination by opportunistic screening in the US.
It is important to consider the following issues in the process of evaluating screening for ages 45 to 49 years:

- Is there a willingness to be screened in this age group? (Adherence)
- Will the current screening tests have equal levels of sensitivity and specificity as those demonstrated for the older population?
- Is the development of CRC in younger ages consistent with the adenoma-carcinoma or serrated pathway, suggesting a potential for bleeding? This is a crucial requirement if FIT screening were recommended.
- Would adenomas or serrated lesions have developed to a large enough size by age 45-49 that colonoscopic evaluation would be beneficial for detection and removal?

Two components of CRC screening can be observed in currently organized systems: the rate of screening of people between ages 45-49 years (a willingness of providers and patients to screen this age group) and the yield of advanced neoplasia detected by screening.

- Given that the American Cancer Society has made a qualified guideline to begin CRC screening at age 45 years, the rate of screening in the 45-49 year age group can be monitored by BRFSS and NHIS, or Hedis measures in integrated health care systems. Partnership with integrated health care organizations such as Kaiser Northern California and Southern California or other prior participants in CRN (network) could be a platform to assess uptake of screening in this age group.
- If there is screening, is the level of detecting adenomas, advanced adenomas, and CRCs high enough to counter the possible risks of colonoscopy?
- Such registries as GI-QUIK (Sp) or CORI registry could include screening versus symptomatic indicators for colonoscopies. The yield of adenomas, serrated polyps, advanced adenomas, or CRC by age group and indication could be captured.
- PROSPR has a registry of colonoscopies (need to check if age 45-49 in their cohort)

The thoughts and potential strategies should also be considered in the research efforts moving ahead.

Dr. Cindy Sears: The Microbiome

I think that this group of young individuals with colorectal cancer (CRC) hold potential promise for adding to our understanding of how the microbiome contributes to CRC and, in parallel, how we might design studies/approaches to interrupting this process. My main ask is that if any of the projected studies/collaborative projects/possible cohorts can create a sample bank for investigation that would be optimal. The sample bank optimally would include oral samples, mucosal biopsies, stool pre-CRC resection (and oral antibiotic exposure) or pre-colonoscopy and plasma. No one group's approach to microbiome analysis is yet optimal—hence the importance of a sample bank to allow a diversity of studies and potential collaborations to evolve. Complementing this with a questionnaire that collects, in particular, diet history and antibiotic exposure, among other CRC risk factors, has merit. I do think this is a place where an investment by Fight CRC/CRI/NIH would have amazing value to multiple investigators.

In my humble (but ardent) view, there is every reason to suspect the microbiome has contributed to the emergence of this deadly disease in the young. The emergence of this parallels the onset of the obesity epidemic in the US population along with the wild overuse of antibiotics. Our antibiotic exposure data which I hope will soon be in press suggests that the antibiotic exposure is at least 10 years before the emergence of the cancer, matching our understanding of the time line from tumor initiation and visualization. I think if we do not weave in the microbiome into studies on young CRC we miss an opportunity and may not get the right composite answers.

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