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BEYOND THE NOISE

CHIP in Liquid Biopsy & Clinical Care



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Executive Summary

On December 5th, 2023 BLOODPAC hosted a virtual webinar on emerging research in clonal hematopoiesis of indeterminate potential (CHIP), including its significance in liquid biopsy data analysis as well as clinical findings. The meeting brought together critical stakeholders including academic researchers, not-for-profits, clinicians, medical device and pharmaceutical companies, and others to share and combine knowledge to advance the field.

The goals of this public virtual workshop were to:

1. Educate BLOODPAC's members and the broader scientific community on the state of the field of CHIP research
2. Identify gaps in knowledge and technology
3. Discover opportunities for BLOODPAC to accelerate progress through collaborative infrastructure and expertise

The outcome of the seminar is to understand if BLOODPAC would like to address these issues under the umbrella of a new CHIP working group.

Over the course of the two-hour seminar, four presentations summarized our current knowledge of CHIP as a cardiovascular disease and heme malignancy risk factor, current challenges and approaches to CHIP classification and subtraction, the role of environmental exposures in the acquisition of CHIP, and clinical perspectives on best practices for management of patients with CHIP.

During the panel discussions, additional experts joined to discuss questions such as:

- How should we handle incidental findings of CHIP mutations in liquid biopsy assays?
- Where and when do we need to be certain we are deconvoluting CHIP from liquid biopsy data?
- What are the clinical implications of incidental findings of CHIP mutations? How should patients be managed, and how does CHIP status impact cancer care?
- What are best practices for CHIP algorithm validation?
- What can BLOODPAC contribute to the CHIP community?

This document provides a high level summary of the discussion, and can serve as a roadmap for BLOODPAC's future contributions in this space. We also hope this white paper will provide insights, best practices, priorities and guiding questions for other organizations in the CHIP space.

Presentations

“We hope that this forum, bringing together clinicians, diagnostic companies, and pharma to consider how we can best serve our patients, will serve to push the field forward and make CHIP a bigger part of the conversation.”

**—DUANE HASSANE, STRATEGIC ADVISOR, ZERO-TO-ONE,
AND FORMER VP, CARDIOLOGY & ONCOLOGY, TEMPUS AI**

CHIP from Epidemiology to Disease Mechanisms in Blood Cancer, Cardiovascular Disease & Beyond

ALEXANDER BICK, MD PHD

Assistant Professor, Medicine, Division of Genetic Medicine, Vanderbilt University

CHIP: INTRODUCTION AND DEFINITIONS

We know, as members of the BLOODPAC community, that our genomes are dynamic and changing entities. Our telomeres change with time, we acquire new somatic mutations, and all sorts of different kinds of cancer can arise. But we also have somatic mutations arising in normal and health tissues, which can cause problems. This field, which include research on clonal hematopoiesis of indeterminate potential (CHIP), is really at the forefront of biology today.

As a starting point, it's important to keep in mind that clonal hematopoiesis means different things to different people. At the most basic level, CHIP refers to a somatic mutation in a blood stem cell which confers a proliferative advantage. But that mutation can actually take a number of different forms. It could be a whole loss of a chromosome, or a point mutation in a myeloid leukemia driver gene, which is how we often define CHIP. Or, the mutation could be in the form of a mosaic chromosomal abnormality, essentially a large scale copy number change or a structural variant. All of these different kinds of genetic changes can cause clonality and ultimately clonal hematopoiesis.

To dig into definitions a little bit more, the World Health Organization's classification of blood cancers just came out in the past year. This edition, the 5th, now formally includes clonal hematopoiesis and several of its subtypes as specific disease entities. In the research community we often define CHIP by several features.

First, we look for the presence of a driver mutation in a gene that causes myeloid blood cancer. This is important because there are also point mutations that can cause lymphoid clonal hematopoiesis and lymphoid malignancies, but these different kinds of clones have a different disease spectrum. Second, for somewhat historical reasons, CHIP is defined as having a variant allele fraction or percentage of the blood of more than 2%. The backstory on this is that when people were studying CHIP around 2014, they were using 100x exome sequencing, and so clones that were smaller than 2% couldn't be reliably detected. But there's nothing really magical about 2%: a 1.9% clone or a 2.1% clone will probably do about the same thing. And finally, the last part of the CHIP definition is that the patient does not have other blood abnormalities, which might suggest some other kind of disease.

CLINICAL ASSOCIATIONS

One of the initial observations was that CHIP is very common in the elderly. Roughly speaking, about 10% of people in their late 60s or early 70s have CHIP. Our research group has been very interested what causes CHIP, and we and others in the field have found both intrinsic factors as well as selective pressures that act on the hematopoietic stem cell as being causes of CHIP. For example, an external cause could be DNA damage from aging, smoking or being exposed to radiation. CHIP has a number of different independently verified associations with other diseases in addition to blood cancer, such as coronary artery disease. And interestingly, there's been a series of papers suggesting that CHIP is associated with development of and worse outcomes in patients with solid tumors.

From my perspective, what all of these associations have in common is inflammation. There's a subset of CHIP driver genes that seem to cause inflammation, and potentially make a number of different pathologies linked to inflammation worse. Now, there's also contrarian views. For example, just a few weeks ago a team at Decode (part of Amgen) reported no evidence that clonal hematopoiesis was associated with cardiovascular disease (Stacey et al., Nat Gen 2023). They're not the only one who have put out papers like this, and usually the devil is in the details of how clonal hematopoiesis is defined. In particular, for this Decode paper, the method of detecting clonal hematopoiesis is able to detect many different kinds of clonal hematopoiesis, not just the myeloid driver kind.

PROGNOSIS AND RISK STRATIFICATION

In general, the field largely agrees that the myeloid malignancy risk of CHIP seems to be different depending on the driver gene, which is why it's so important to understand how CHIP is being defined in a given data set. In particular, DNMT3A, which is a methyl transferase and perhaps the most common CHIP gene seen in about half of patients, has very little increased risk of myeloid malignancy. In contrast, if you start looking at genes involved in splicing, or genes that are signaling molecules like RUNX1, these kinds of genes have markedly increased risk of malignancy.

The other factor that has been very consistently observed is that the size of the clone matters. A really large CHIP clone that makes up more than 20% of a patient's allele fraction confers a markedly increased risk of myeloid cancer compared to individuals who have very, very small clones.

Several researchers in the field have tried to develop CHIP risk scores to integrate across these different factors. Using these calculators, we've found that high risk individuals are actually a very small fraction of CHIP, perhaps less than 1%. About 10% of individuals might be at intermediate risk, and about 90% of individuals are at low risk. However, individuals who have particularly high risk features have a 50% chance of progressing from CHIP to frank malignancy over five to 10 years. And so this might where we think about intervening therapeutically, although there are no FDA approved therapies. However, there are active clinical trials using different strategies to either target the mutations or to target the marrow environment. And I suspect over the next year, we'll start to see additional trials in this space.

SUMMARY

- CHIP is generally defined as the presence of a clonally expanded hematopoietic stem cell population at a VAF $\geq 2\%$ in individuals without evidence of hematologic malignancy, dysplasia, or cytopenia
- Incidence of CHIP increases with age and affects an estimated 10% of individuals over age 70
- Driver mutations underlying CHIP have been identified, with a majority of involved genes related to DNA methylation, epigenetic modification, and inflammatory responses
- CHIP is associated with a 30-40% increase in all-cause mortality, mainly driven by increased incidence of atherosclerotic heart disease
- Individuals with CHIP have an increased risk of developing a hematological malignancy
- High risk individuals make up less than 1% of CHIP patients, but individuals who have high risk features have a 50% chance of progressing from CHIP to frank malignancy over five to 10 years

Bioinformatics Strategies for Identifying Clonal Hematopoiesis in NGS Liquid Biopsy

ANNE SONNENSCHN, PHD

Senior Bioinformatics Scientist, Tempus

CHIP CLASSIFICATION METHODS

In this segment, we're going to focus on CHIP as noise rather than CHIP "beyond the noise". At Tempus, we've been working on methods to screen CHIP variants in liquid biopsy testing for cancer patients. Targeted sequencing of plasma for circulating tumor DNA is a very noninvasive method for precision medicine, and it's becoming increasingly sensitive. These assays frequently screen for small variants, SNPs and indels. At Tempus, we have a number of different liquid biopsy assays. The main one I'm focusing on today is our xF Plus assay, which can detect variants down to 0.1% variant allele fraction (VAF). I'll also briefly be mentioning the xF Monitor, which is an algorithm for estimating tumor fraction in plasma samples. And then I'll be using some data from xT, which is our solid tumor assay. This is a sequencing assay for solid tumor biopsies usually paired with matched healthy blood from the same patient.

In addition to being medically and scientifically relevant in its own right, CHIP is a major confound for this type of testing. This is most saliently the case with the gene TP53, the most frequently mutated pan-cancer gene, which is less common as a CHIP driver but still occurs at an appreciable frequency. The degree to which this is a confound is heavily dependent on the depth of sequencing. When you start using MRD liquid biopsy assays with a sensitivity of 0.1% VAF, CHIP mutations are ubiquitous in healthy adults: very close to 100% of the people you sequence will have a detectable CHIP clone at that level.

The gold standard for identifying CHIP and distinguishing it from tumor variants is having matched white blood cell sequencing data. CHIP variants will have approximately equal fractions in plasma and white blood cells, unlike tumor-specific variants. Relative absence of putative CHIP variants in tumor samples is another really strong piece of evidence. Tumor variants are going to be enriched in the solid tumor, although CHIP variants may also be detectable in the solid tumor if there's immune infiltration, but usually not to the same degree. In addition, CHIP variants fluctuate longitudinally quite a bit less. So if you have multiple time points from patients undergoing treatment, you do expect your tumor variants to decrease over time, hopefully, while your CHIP variants are not necessarily going to respond to treatment or respond in the same way.

CLASSIFICATION WITHOUT PAIRED SEQUENCING

However, how do you identify CHIP if you don't have white blood cell or solid tumor sequencing data available? There are a number of different features that can be used for identifying CHIP in this case.

The most standard method is just look at the gene. CHIP drivers are overwhelmingly in DNMT3A, and if you extend that out to the five most common CHIP genes, you correctly classify most variants: about 83% in a Tempus dataset of over 600 pathogenic variants. Notably, however, this is not balanced performance because although we correctly classified 98% of the tumor variants as tumor, we only correctly classified two thirds of the CHIP variants. Although this is pretty good performance, in terms of actual utility we need to do better.

A slightly more sophisticated approach people will take is to look at the historical prevalence of individual variants. So, has this variant been seen in heme samples? Has it been seen in lymph tissue? Is it a blood cancer variant, or is this most commonly associated with solid tumors, specifically, the tumor type that this patient has? Unfortunately, this doesn't work well for TP53. At Tempus, 75% of the time we see this mutation it's tumor-associated, and 25% of the time it's actually CHIP. However, using this approach we do correctly exclude about 90% of the CHIP variants.

A final approach, which is used in our xF monitor algorithm, takes into account the allele fractions of the tumor variants to propose a tumor fraction estimate for a given plasma sample. As I previously mentioned, you expect your tumor variants to be similar allele fractions to each other, but you expect your CHIP variants to potentially be outliers. In the the xF algorithm, tumor variants correlate quite strongly with this tumor fraction estimate but CHIP variants do not. Using a model trained on a set of 600 variants, and completely naive to gene or variant historical prevalence, we accurately classified about 74% of both the tumor and CHIP variants. The reason this approach is not 100% are the variants that are low allele fraction in samples with low tumor fraction, which is unfortunately very common for CHIP.

Another feature that has attracted a fair amount of excitement recently is the use of fragment size of sequencing reads that contain tumor variants in plasma. Tumor-derived cell free DNA has a very distinctive fragment size distribution, while CHIP variants look much more like germline variants in terms of size.

We've discussed three categories of features that are all independently quite strong and totally orthogonal. At Tempus, we've combined all three in one ensemble model and are beating 90% accuracy (this work has been submitted as an abstract for AACR). I would note, though, that we're not quite at parity with an approach that's solid tumor informed or white blood cell informed, where accuracy is close to 100%. Part of this is probably because there are some challenging variants that are very difficult to classify with any of these features.

In terms of what the field needs to advance plasma-based classification, gold standard reference sets for plasma model development are critical. The other big question we're thinking about is, what do we do when CHIP is detected in a liquid biopsy assay? What level of accuracy do we need before informing physicians and patients? Do we think physicians will find this relevant or useful?

SUMMARY

- Several features can be used to classify CHIP mutations in solid tumor liquid biopsy data, including relative enrichment in blood vs tumor samples, longitudinal fluctuation, historical prevalence of individual variants, and fragment size of sequencing reads
- Tempus has developed a novel algorithm that estimates tumor fraction for a given plasma sample, which is then used to identify CHIP mutations on assumption tumor variants, but not CHIP mutations, should correlate quite strongly with this tumor fraction estimate

Clonal Hematopoiesis in Solid Tumor Patients: Implications for Secondary Malignancies & Diagnostics

AHMET ZEHIR, PHD

Executive Director, Precision Medicine Diagnostic Innovation, AstraZeneca

CANCER TREATMENT AND CHIP

My focus in today's talk is going to be CHIP in solid tumor cancer patients. At Memorial Sloan Kettering Cancer Center, we developed and implemented a next generation sequencing based diagnostic assay called MSK Impact that enabled us to identify mutations, copy number alterations, and structural variants in over 500 genes. In this assay we sequence both the tumor and the matched white blood sample, which really enabled us to definitively identify somatic vs. germline alterations. Around 2015, when the seminal papers on CHIP started to appear, we took about 9000 patient samples and identified the somatic mutations in the blood and saw that about quarter of the patients had clonal hematopoiesis. We were able to identify smoking and prior radiation therapy as factors that are associated with presence of CHIP, and we also further showed that presence of CHIP increases the risk of developing secondary malignancies.

These findings lead to the establishment of the first clonal hematopoiesis clinic in the world, and we started prospectively reporting CHIP mutations to our clinicians and to patients. We also looked at how oncologic therapy shapes clonal hematopoiesis in cancer patients. For this, we created a cohort of almost 25,000 patients with deep phenotypic data and found that radionuclide treatment, external beam radiation and cytotoxic therapy were associated with CHIP, but targeted therapy and immunotherapy were not.

We were also lucky enough to find 525 patients for whom we had two different blood draw time points to ask the question: does treatment promote pre-existing CHIP or induce new mutations (Zehir et al., Nat Med 2017)? We also wondered how treatment changes the allele frequencies or growth dynamics of CHIP mutations. The answer to first question is that about 95% of the CHIP variants were detected at both time points. However, in cytotoxic therapy and radiation-treated patients, we saw a vast majority of mutations going up in allele frequency over time. In contrast, targeted therapy and immunotherapy-treated patients had a pattern very similar to untreated patients.

CONFOUNDING EFFECTS OF CHIP IN TUMOR DIAGNOSTICS

Another question I want to talk about is the implications of CHIP mutations in tumor diagnostics. I'll start with a study that we did looking at the confounding effects of CHIP mutations in solid tumor patients. In this instance, we had a cohort of about 18,000 patients where we asked, if we didn't have the blood sample, how many of these mutations we would misclassified as somatic tumor mutations? We found that up to about 5% of the patients had CHIP mutations mis-diagnosed as solid tumors, which is a not insignificant number. Furthermore, most of these mutations were absent from population databases and over half of them were labeled as oncogenic, or likely oncogenic, in the oncoDB database, suggesting that if you see this mutation you're probably going to report it.

Now to quickly touch upon liquid biopsies: utilizing white blood cell sequencing data, we can definitively identify what mutations are originating from what tissue. In a cohort of 617 plasma and white blood cell pairs sequenced using the MSK Access assay, we found that we can cluster mutations into four groups based on allele frequencies in plasma and in white blood cells (Brannon et al., Nat Comms 2021). For mutations with high allele fraction in both plasma and white blood cells, we can show that up to 77% of CHIP variants were removed through use of white blood cell data. And there are hundreds, if not 1000s, of mutations, really making it hard to classify correctly without having white blood cell data.

And lastly, I'll touch upon a study that came out earlier in the year utilizing data from about 1400 SNPs (Fairchild et al., STM 2023). The authors generated a list of variables and trained a logistic regression and Random Forest classifier to differentiate CHIP mutations from tumor mutations. The overall success was about 90%, and what was interesting to me is that allele frequency seem to be the most important variable for both of the machine learning methods used, followed by gene name and the mutation signatures.

And I'll conclude with with a few big-picture questions for our community. We talked about using CHIP as a biomarker to identify high risk cancer patients. How do we do this? How would this look in practice and can we treat these patients prophylactically? What is the most optimal way to identify and remove CHIP variants in liquid biopsies in the absence of WBC sequencing?

SUMMARY

- Cancer treatment selectively increases CHIP prevalence in cytotoxic therapy and radiation-treated patients
- The profound selection pressures of chemotherapy and radiation may drive the emergence of clonal hematopoietic populations, while targeted therapy and immunotherapy did not elicit a similar response
- The confounding effects of CHIP mutations in solid tumor patients can be significant: in one study, up to 5% of patients had CHIP mutations mis-diagnosed as solid tumors
- A significant minority of CHIP mutations are very hard to classify correctly without paired white blood cell data

Clinical Implications of Clonal Hematopoiesis

MARY BROPHY, MD

Assistant Professor, Hematology & Oncology
Boston University

CHIP AS A BIOMARKER

Hematopoietic stem cells are a rapidly proliferating cell type which, as a normal part of aging, develop mutations leading to clonal hematopoiesis. We know that CHIP is associated with a decreased overall survival, increased risk of metabolic malignancies and cardiovascular complications compared to age-matched controls. But if we step back and look at this, the increased mortality is being driven by cardiovascular events: clinically, older patients with CHIP are dying of other causes, often cardiovascular in etiology. We also know that CHIP is associated with an increased risk of transformation to myeloid malignancy. But again, if you look at it on the clinical side, only a small fraction of individuals with CHIP will develop these hematologic malignancies: the risk of evolving is about 0.5 to 1% per year. Clonal hematopoiesis risk scores have been developed which show that the overwhelming majority of patients with CHIP are at low risk for transformation.

So, CHIP appears to be a biomarker in the myeloid series that gives some idea of a risk for developing malignancy and death. However, if we compare CHIP to lymphoid proliferative disorders, such as monoclonal gammopathy of undetermined significance, which has a prevalence identical to what we see with CHIP, we have learned from 40 years of monitoring that the improvement in survival and morbidity is only seen in those patients who have progressed to the malignancy. And it's unclear whether that benefit is really coming from early detection. And so, what's the potential return on investment in testing for CHIP clinically?

We know that individuals can have a higher risk for developing CHIP independent of age based on exposure to factors such as cytotoxic chemotherapy and ionizing radiation. Clinically, we need to ask: are we going to move towards screening these high-risk patients for CHIP? What other types of exposures do we need to start tracking? For example, coming from the VA, the military toxic exposure issue is really exploding right now. Patients ask, what's the impact on my health? And what are you doing about it? I think there's a lot of discovery that can be done in understanding these exposures. Petroleum, benzene, radiation—these are things that the people in the military are exposed to at much higher rates, and we need to determine if warfighters are developing earlier or higher-risk CHIP. In addition, we need to parallel this discovery work with therapeutic research to halt or revert what could be progression to myeloid malignancy as we begin to discover these patients.

DRUG DEVELOPMENT FOR CHIP

With respect to drug development for CHIP, one of the main challenges I see is that many of the CHIP driver mutations are loss-of-function or epigenetic/transcription-related genes, which we already know are difficult to drug. We also have to be thinking about the unknown effects of treating CHIP. For instance, what if the clone we target has been “suppressing” the expansion of other clones, which could then begin to proliferate post-treatment? The paradigm here that many of us fall back to clinically is the case of chronic myelogenous leukemia. This was a universally fatal disorder when I was starting hematology training, and after the discovery of a genetic marker and development of a targeted therapy it’s simply a matter of taking a pill to keep the disease under control. Many of us hope that we’re heading in that direction with CHIP moving forward.

And finally, understanding the causality between cardiovascular disease and CHIP is critical as we move to treat patients. Is CHIP just a background association, no different from the inflammatory markers that we already know exist? Or is it a driver? And if so, is there any reversibility? As a clinician, I want to be able to know that I am doing the right tests and that I know what to do with the results. We have to know these tests are accurate, how to implement them within our healthcare system, and how to communicate risk to our patients. We need to know which CHIP mutations are actionable and why, and how we can best assist our patients. In sum, you can’t have a good biomarker unless you have validated it and implemented it on the clinical side, which includes creating the knowledge needed for clinicians to sit across from their patients and give them their prognosis.

SUMMARY

- The driver mutations underlying CHIP are involved in modulation of inflammation, which may underlie the association with cardiovascular disease
- Individuals can have a higher risk for developing CHIP independent of age based on exposure to factors such as cytotoxic chemotherapy and ionizing radiation
- The military population is exposed to potential CHIP-driving environmental factors, making this an important patient group to begin screening once the clinical implications of a CHIP diagnosis are better understood
- While drug development for CHIP is ongoing, we must be wary of the unknown effects of suppressing CHIP clones
- CHIP as a biomarker has a significant way to go before we are equipped to communicate prognosis and recommended treatments/interventions to patients

Panel Discussion and Q&A

“Data availability is a foundational concept at BLOODPAC, and it's had a tremendous impact in bringing liquid biopsy assays closer to the clinic.

We would love to bring this approach to the CHIP space, working collaboratively on projects such as best practices in CHIP classification methods, contributions by industry partners of matched tissue data, and the creation of a normal negative control dataset.”

**—JIM GODSEY, VICE PRESIDENT, MOLECULAR
GENOMICS & ONCOLOGY R&D, QUEST DIAGNOSTICS**

Panelists

MODERATOR: JIM GODSEY

VP, R&D and CSO, Molecular Genomics & Oncology
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JUSTIN FINKLE, PHD

Senior Bioinformatics Scientist
Precede Bio

ROBERT GROSSMAN, PHD

Director, Center for Translational Data Science
University of Chicago

SAKSHI JASRA, MD

Assistant Professor, Department of Hematology and Oncology
University of Vermont

MARISA JUNTILLA, MD PHD

Laboratory and Medical Director
Guardant Health

Q1: Our first question is for Marisa. Where and when do we need to be certain we are deconvoluting CHIP from our clinical ctDNA data? What are the most commonly used methods for accomplishing this task, and do different intended uses dictate different methods?

MARISSA JUNTILLA (GUARDANT)

I do agree that there should be some CHIP filtering because we see a lot of variance, especially in the elderly and anyone who has been exposed to environmental stress or chemotherapy, which is obviously the patient population that is served by liquid biopsy in solid tumor testing. Two of the big areas that are thought about here with solid tumor CHIP filtering are algorithmic based approaches and buffy coat/PBMC-based approaches. I believe that both of these approaches should be part of the path of assay development. You will most likely need to do buffy coat sequencing at some point during your development, and move towards an algorithmic approach when you've developed enough of a database to understand what CHIP looks like in your assay.

In addition, developing an algorithm requires large datasets and varied patient statuses, and so this is not always accessible to smaller laboratories. In these cases, buffy coat sequencing may be the way to go to filter out CHIP. I do want to also note that the tissue information that is important for CHIP filtering is not always available in a liquid biopsy setting. So all of those all of those approaches will require some type of plasma-only approach in my opinion.

Speaking to the other part of your question, in solid tumor biology we obviously see different different driver mutations from the hematologic mutations that are characteristic of CHIP. Therefore, in solid tumor mutation testing for therapy selection, CHIP is not so much of an issue. But in the other settings such as MRD, or therapeutic monitoring, you may need to have a more sophisticated CHIP filter, or add on additional layers such as a methylation-based approach or depart from mutation-based signatures.

Q2: Thank you Marisa! The next question is for Justin Finkle. Justin, you previously worked on leveraging CHIP to discover novel age-related drug targets at TenSixteen Bio. Can you briefly explain how this worked? Interestingly, it seems that your work would require that we must maintain access to CHIP data?

JUSTIN FINKLE (PRECEDE BIO)

At TenSixteen Bio, we had a great team working on identifying and understanding CHIP biology, which is the flip side of removing it as a noisy variant. One of the first steps underpinning this work is the question of how we reliably and sensitively detect CHIP variants. There are a few layers to this: for example, what is our definition of a CHIP variant? How do we distinguish it in a liquid setting? And then how do we categorize and mix that with clinical information? And so, at TenSixteen, we went through this process in a number of different datasets, some of our own proprietary work as well as in some publicly available datasets like the UK Biobank. We were really focused on identifying the shift to hematologic malignancy in individuals and determining therapeutically relevant indications.

Dr. Bick, would you like to weigh in on that a little bit?

ALEX BICK (VANDERBILT)

I would just say that you can think about targeting the driver mutation in CHIP or you can think about targeting something else. And that something else could be, for example, inflammation, if you're thinking that inflammation might be contributing to the clone expanding, or that inflammation is leading to heart disease, liver disease, and whatever other sort of effects that the CHIP clone is producing. And so I think some of the clinical trials that are going to launch in the next year are designed to test these exciting hypotheses. The only way to test them is by doing the clinical trial, because we've already done it in mouse and we've looked at the historical data, but ultimately we need a randomized trial for some of these questions.

Q3: Thank you Justin and Alex! This next series of questions are for Sakshi Jasra. How should we handle incidental findings of CHIP mutations in liquid biopsy assays? Should we report it or not? Do physicians want this info and is it actionable?

SAKSHI JASRA (UNIVERSITY OF VERMONT)

In order to be able to answer those questions, we need to remind ourselves that we're really talking about two different groups of patients with clonal hematopoiesis. We're more commonly talking about the patients with a known malignancy, which we tend to think of as solid tumors, but certainly, could be malignancies like lymphomas, and we're conducting liquid biopsies as a way of either diagnosing or looking for an actionable mutation. Then we have the other group of patients, which is what we had explored in our world trade center cohort, where we think that either they're healthy and have no clonal or other cytopenias or for whatever reason, if there's been an environmental exposure or an occupational exposures such as for our veterans, we think that they may have an increased risk of developing a malignancy and potentially even CHIP at baseline. The approaches for each of these two groups of patients can potentially differ.

I'll start with your first question, of whether incidental CHIP findings should be reported to me as a physician. In my opinion, there's a plethora of data showing that there is an impact on overall survival. We know that these patients have an inferior overall survival, we know that there's an impact on cardiovascular mortality. So yes, we definitely should be reporting CHIP. The question of how we should report CHIP is a little more difficult to answer. It depends on how the testing is conducted, whether there paired tumor and white blood cell DNA testing gets done, and how accurately we're actually reporting these variants. Reporting of the CHIP mutations will allow the clinicians to be able to monitor them in a longitudinal manner. I love what Mary said earlier that the blood is the easiest organ to sample. You can do it every day if you really wanted to determine if there is clonal evolution and if these patients develop cytopenias over time.

The really difficult question, in my opinion, is whether incidental CHIP findings are actionable. I would say that even though we have data to show that these patients have inferior overall survival, all of the studies actually show that patients are more likely to die from progression of their underlying malignancy rather than clonal hematopoiesis. So the risk of actually dying from CHIP is very, very low. And the risk of the absolute risk of developing the malignancy is again on the order of about 0.5 to 1%. Though why this occurs, I think, again, still needs to be parsed out. It could be the hyperinflammatory phenotype of clonal hematopoiesis, or CHIP could serve as a sort of a biological marker for fitness in the patient.

In order to be able to move this field forward, what we really need are two things. First, we need to define what constitutes high risk clonal hematopoiesis. Some groups have proposed definitions for high risk such as having more than two mutations in the canonical CHIP genes, but there's no consensus. Second, I think validating and constructing the CHIP risk calculator in our cancer population would be a really great step to be able to provide clinicians with the data that they need to counsel patients and really empower them to have fruitful discussions.

But to say that we should be modulating our adjuvant treatment in our cancer population based on the presence of CHIP I think is a little premature, especially if we have data to show that the patients are more likely to have progression of their malignancy rather than true therapy-related neoplasms.

Mary, would you like to weigh with a couple of comments on that?

MARY BROPHY (BOSTON VA)

I would just add that I completely agree with the need for a validated risk score and a better understanding of the biology underlying CHIP and how patients progress to malignancy. We need to take into consideration that these patients that have tumors have already declared themselves as having malignancies and the clonal hematopoiesis is in the background. Its relationship to the existing malignancy, even if hematologic, is unclear-- we still have lots of discovery research to do on this topic. I do agree also that we do need to inform patients of incidental CHIP findings, but we need to do it in a way that de-escalates. The usual situation of I have is of a patient being told told they have the immunophenotype of CLL [chronic lymphocytic leukemia]. And the patient and their family comes in the door expecting that they're going to be told they're dying, but you have to walk it back and convey that this is an early marker. That's not an uncommon feature in clinic when these biomarkers are out there and being used.

Q4: Thank you both. And finally, a question for Bob Grossman. Bob, as a direct result of this New Frontiers presentation on CHIP, what are your thoughts on BLOODPAC's ability to create a repository for CHIP data within the existing BLOODPAC Data Commons?

BOB GROSSMAN (UCHICAGO AND OPEN COMMONS CONSORTIUM)

This is clearly an area of important interest for the BLOODPAC community. From a technical viewpoint, it would be straightforward to bring in CHIP data to the BLOODPAC Data Commons and interoperate with other types of data platforms out there, if there was member interest in that. There are a number of simple things we could do, as there's no standard location for community datasets around CHIP data and the BLOODPAC Data Commons could naturally fill that role. This would be especially important for environmental exposure data, as Mary Brophy and other speakers have talked, where longitudinal datasets are lacking. In other areas, we've worked as members pre-competitively to agree on minimum technical data elements to for liquid biopsy data collection and we could do that here. In addition, establishing a repository of member-contributed data may be beneficial for initiatives such as CHIP risk scores which require a critical mass of data. One of the strengths of BLOODPAC has always been the fact that individual members come up with ideas and projects that we work on, and nothing is done without a consensus. The concept of a BLOODPAC-maintained CHIP repository is a very exciting opportunity, and I was very impressed by the talks I heard and the potential in this area.

Audience Q&A

This question from the audience is for Alex Bick. Alex, are we at a stage where thinking about the societal cost of testing for CHIP makes sense in terms of excess risk of cardiovascular events and heme malignancies? And, with the caveat that not all associations are necessarily causal, are their gains to be had in drugging CHIP? And as we think about drug development, are there any considerations that we need to think about in designing clinical child trials for this topic?

ALEX BICK (VANDERBILT)

That's a great question, and I'd like to spend the next two hours addressing in detail! I think the key to this question is applying the paradigm of precision medicine, which is what BLOODPAC has been working towards on the solid tumor side. If you can identify a small enough subset of patients that have a high enough percentage of risk, and treat them with a drug where the benefit outweighs the harm, then I think the answer is absolutely, yes. But next question is, are we there yet? Or, how close are we to that situation? I think the place where we'll see benefit first is in the nearly MDS [myelodysplastic syndromes] space, where we have patients who are at very high risk for transformation, and may even have disease burden, or will soon have disease burden from susceptibility to infection. If there's a highly targeted therapy that works well to address the specific mutation, or even if it's an anti-inflammatory drug that is reasonably well tolerated, I think that's probably where we'll start with trying to define these clinical trials. Efforts like BLOODPAC are crucial in finding those patients. Matching the right patient with the right clinical trial for something that we're not really routinely testing for, is a big challenge to overcome. That's why I'm really excited about the thought of a Data Commons where, even if you don't know who the patients are, at least you know that such patients exist and the prevalence of a given mutation is at a certain level. This type of information is going to be really critical to the field moving forward.

This question comes from a member of the FDA. Can the speakers comment on the potential of the CHIP signal interfering with MRD [molecular residual disease] assay findings? For example, given that buffy coat sequencing is relatively expensive to perform, some companies are using lower sequencing depth, such as 150x. Is that a high enough depth? What about algorithm validation for CHIP signal removal? More generally, what are best practices for dealing with CHIP contamination of MRD signal?

DUANE HASSANE

I think this question is addressed in studies like TRACERx, in which the false positive rate of recurrence gives you an idea of whether the thresholds that companies in the space are using for their background are sufficient. In defense of the tumor-informed MRD companies, I believe they have been very proactive about CHIP.

DON JOHANN (UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES)

I'd like to add that with the new developments in sequencing technology, such as Illumina's new Novaseq X Plus platform, the cost of sequencing has dropped by as much as 80%. For instance, previously I would sequence a whole genome here in Little Rock, Arkansas, at 30x coverage for \$1,000. With the Novaseq X Plus, I can now do the same thing for \$200. This new platform can also give us longer read lengths, which can provide a lot more specificity with alignments. So that could change everything, and there's nothing like more data in this type of application to convince yourself statistically about what you're seeing.

MARISA JUNTILLA (GUARDANT)

Yes, I agree, we need more data. As Ahmet mentioned, we can also depart from the variants as our method of detection and use methylation signatures, which can also be trained on the tumor-of-origin type of assays, and that could help alleviate that pressure on CHIP. As I mentioned before, although CHIP is seen throughout the genome, it's not well characterized within the driver genes in solid tumors that we are using for MRD. And for the liquid tumors that have MRD analysis, we have the antigen receptors to follow.

AHMET ZEHIR (ASTRAZENECA)

I think this question could be a very important topic for BLOODPAC to pick up on and work towards harmonizing methodologies and datasets being generated. We talk a lot internally around how CHIP might be confounding molecular response results and MRD results. And, you know, it becomes an expensive activity to sequence the buffy code. But that seems to be currently the best way of handling CHIP variants. But the challenge now is, how do we move on to actually using algorithms to identify these mutations and removing them with high levels of confidence so that we know that data that we're getting back from patients in these trials is actually going to be useful?

References

Stacey, S.N. et al. Genetics and epidemiology of mutational barcode-defined clonal hematopoiesis. *Nat Genet* 55, 2149–2159 (2023). <https://doi.org/10.1038/s41588-023-01555-z>

Zehir A. et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med*, Jun;23(6):703-713 (2017). <https://doi.org/10.1038/nm.4333>.

Brannon, A.R. et al. Enhanced specificity of clinical high-sensitivity tumor mutation profiling in cell-free DNA via paired normal sequencing using MSK-ACCESS. *Nat Comm* 12, 3770 (2021). <https://doi.org/10.1038/s41467-021-24109-5>

Fairchild L. et al. Clonal hematopoiesis detection in patients with cancer using cell-free DNA sequencing. *Sci Transl Med*. Mar 29;15(689):eabm8729 (2023). <https://doi.org/10.1126/scitranslmed.abm8729>.

