Session 2

Mutational discordance: the big challenge in personalized treatments – any solutions?

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Published in:
Colorectal Disease

DOI (link to publication from Publisher):
10.1111/codi.14079

Publication date:
2018

Document Version
Accepted author manuscript, peer reviewed version

Session 2: Are we treating the correct patients?

Mutational discordance: the big challenge in personalised treatments - any solutions?

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Disclosures

The authors have no conflict of interest.

Word count excluding abstract, references, tables, figures and legends

1397

Abstract

The great challenge for oncologists treating patients developing or progressing with metastatic disease is to be able to offer a truly personalised and targeted therapy that can have an early and meaningful effect on the course of the disease. At present, the known molecular markers are limited in their frequency and reliability in determining the use of newer chemotherapies. Prof Eng discusses the challenges faced in ensuring timely and effective treatments based on the molecular profile of the tumour and discussed the potential role of real time analysis of mutational changes in the tumour when progression occurs.

I have been asked to discuss mutational discordance, the challenge in personalised therapies and is there a potential solution for our patients?

There have been some new drugs that have been approved recently; many of these are variants of anti-VEGF and anti-EGFR therapies. We have also noticed the development of molecular markers and their role in the treatment for our patients. But regardless, the overall survival for surgically ‘unresectable’ patients is still very poor with a reported 5-year survival of 13%. The TRIBE trial was recently updated indicating that the 5-year survival was 25% if one uses FOLFOXIRI and Bevacizumab[1], but obviously this is only feasible in select patients. So we have to look for other treatment options. As a result we must enrol patients onto clinical trials.

Historically CEA was used to detect tumour recurrence or progression but unfortunately 15-30% of patients with tumour recurrence or progression do not have an elevated CEA. The best way to evaluate these patients is by diagnostic imaging, clinical benefit and additional blood tests. However as medical oncologists we are faced with what the patient is personally experiencing. Patients want to get started on treatment as soon as possible, especially if they are being treated for palliative purposes. They are often unwilling to wait for their tissue molecular marker analysis, which may be a minimum of 7-10 business days, or if not longer, should their tissue biopsy reside in a warehouse.

In the USA the majority of oncologists start their patients on Bevacizumab as a biologic agent often because the patient is unwilling to wait. We obviously have our own anxiety, especially when we see young patients who we want to initiate treatment for as soon as possible, patients with a high degree of tumour burden and/or those with the BRAF mutation tumour type that's traditionally known to be a poor prognostic factor. Historically, we know that the first regimen is usually the one that has the largest impact on the patient in regards to overall survival.

Thus far we have no molecular markers for anti-VEGF therapy that are predictive markers for benefit of therapy but we know it is imperative to test for the KRAS tumour mutation status when considering anti-EGFR therapy[2], and we know that the BRAF mutation is an extremely poor prognostic marker[3].

The PRIME trial saw our approach to the KRAS mutation include extended RAS analysis[4]. The KRAS mutation is present in about in 30-50% patients, but even with a KRAS wild-type (WT) tumour, there are about 15-20% additional RAS mutations which will impact patient care when we are considering anti-EGFR therapy.

The FIRE-3 study compared FOLFIRI + Cetuximab vs. FOLFIRI + Bevacizumab with a primary endpoint of response rate[5]. An extended RAS analysis was completed and noted an overall improvement in overall survival if you provided anti-EGFR therapy to an all RAS WT patient. What I think is very important about this study is

that the BRAF mutant patients had extremely poor median OS regardless of the the regimen provided. CALGB 80405 contradicts FIRE 3 study (unpublished data), when comparing FOLFOX or FOLFIRI with randomization to bevacizumab based therapy vs. cetuximab based therapy with essentially equivalent overall survival for both arms. Based upon these trials we now know that the KRAS mutation is not the only molecular marker that we should be considering. We must also consider extended RAS analysis and the BRAF mutation.

Limitations

There are limitations in regard to tumour mutation analysis. It can be very difficult to obtain the original archival tissue especially if it resides in a warehouse or if it was obtained several years ago. Regarding the primary tumour, sometimes the amount of tissue is insufficient and the storage conditions obviously can impact upon tissue analysis. The potential for discordance of the primary and metastatic tumour is a concern, although traditionally, as mentioned earlier, KRAS has very high concordance (>90%)[3]. You may recall the New England Journal of Medicine paper (2012) which presented a renal cell carcinoma patient which had significant intra-tumoural heterogeneity within one patient[6].

Another option is a fresh tissue biopsy, but this is results in additional time and cost associated with another invasive procedure, including scheduling the procedure and deciding upon which metastatic site to biopsy.

There is high concordance for KRAS, BRAF and PIK3CA but mild to moderate discordance for PTEN and CMET. Although CMET has not found a role yet in colorectal cancer, it appears to be a late event. So it is reasonable to ask what other options we have.

Future potential options

One of the potential roles for an evaluation is the so-called liquid biopsy. This is a non-invasive alternative to tumour mutation analysis that allows real-time capture of biologic changes within the patient and may be more representative of the current tumour mutation state allowing us potentially to detect the development of treatment resistance.

We have all seen the KRAS WT type patient who started on anti-EGFR therapy and then eventually progress. There have been small studies that have indicated that the mutation status may change in associated with progression. Is it potentially quicker? Obviously that would be very helpful and may impact the initial treatment approach; it may change the way we perform diagnostic imaging and the timeframes to when we evaluate our patients and especially beneficial when we have a patient with a normal CEA. Could this be potentially more cost effective than what we are currently doing?

Fresh tissue biopsies are expensive. When considering circulating free DNA, it is not only elevated in cancer patients but is also elevated in inflammation, trauma and sepsis. But we do know that circulating free DNA is 4-5 times higher in cancer

patients compared to controls due to either secretion, apoptosis or necrosis[7]. We can also detect other point mutations, copy number variations and structural rearrangements. This is very helpful in the setting when a patient has had a metastatic resection, especially if we could find a non-invasive approach to detect early relapse instead of just relying on diagnostic imaging. Obviously if the patient is currently on treatment (for example anti-EGFR therapy), use of cfDNA may indicate early signs of resistance rather relying on diagnostic imaging. Could we possibly use this as another alternative?

There are limitations to liquid biopsy at this time. There's still some debate as to whether you use serum versus plasma although the majority of studies have suggested plasma is better [8]. There's still some variability in DNA extraction and this may result in a variability of about 50% between yields. It's very important to capture all the DNA fractions and obviously the smaller fractions may be the most informative from the primary and metastatic site. Currently, there is no overall consensus about storage or approach to these samples. There are some very recent publications indicating there is significant variation in technique and the majority of studies have a sensitivity of 5% or less although BEAMing is more sensitive. BEAMING isolates DNA and then amplified using magnetic beads, undergoes PCR, flow cytometry and then it quantitates the mutant versus wild type. Hotspot mutations are identified in specified genes so it has a sensitivity of less than 0.1%. Therefore, you can identify one mutant allele in 10000 WT alleles.

In conclusion liquid biopsy is very promising, but is still not standardized, and needs to be validated in large prospective studies and to be made widely available at a reasonable cost. Obviously this may vary based upon technique and currently at this time we would still recommend using cfDNA in conjunction with diagnostic imaging. cfDNA is not considered a standard of care, but it is definitely intriguing.

Summary of the key points

- Currently there are no predictive markers for the benefit of anti-VEGF therapy.
- It is imperative to test for the KRAS tumour mutation status when considering anti-EGFR therapy
- BRAF mutation is an extremely poor prognostic marker.
- Extended RAS analysis should also be considered.
- The potential for discordance of the primary and metastatic tumour is a concern, although traditionally KRAS has very high concordance (>90%).
- Small studies have indicated that the patient's mutation status may change with progression.
- Use of cfDNA may indicate early signs of resistance rather relying on diagnostic imaging.
- Liquid biopsy is very promising, but is still not standardized, and needs to be validated in large prospective studies and to be made widely available at a reasonable cost.

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