So thank you Arunhara and Pratik for inviting me.
Good afternoon all.
My lecture is not about data.
This is a very didactic lecture on biomarkers for immunotherapy.
The established and the emerging ones.
And I start with this wheel of hallmarks of cancer.
And of the several hallmarks of cancer, immunization, genomic instability and tumor promoting inflammation
are the foundational principle of the immunotherapy.
And they also guide us in biomarker selection and biomarker discovery.
So how do these three cooperate with each other to overwhelm or subvert the immune surveillance process?
So the first is that through the molecular progression and molecular mimicry, they create
antigens which are not unique and which are not immunogenic.
The second way is the APC fails to educate the cytotoxic tel lymphocytes and fail to
educate the cytotoxic tel lymphocytes because of the inefficient antigen presentation again
through the molecular phenomena leading to loss of function alterations in HLA, class
1 and beta 2 microglobulins.
And also through activation of CTMA4, CD8086, synapse which leads to an activation of the
cytotoxic tel lymphocytes.
The cytotoxic tel lymphocytes can also be made energy in the effector site by the PD-1, PD-1
and X is activation or similar activations in other co-innovation signaling.
And lastly through the cluttering of the microenvironment with immunosuppressive cells.
And to overcome this ineffective immunosurvalence, several strategies have been used.
The four most, the one which has given us the most significant results and has been transformative is use of immune checkpoint inhibitors which block those checkpoints.
So we interpose a anti-CTL4 antibody that will prevent the synapse of CTL4 with CD8086 or
use anti-PD-1 or PD-L1 antibody that will prevent the synapse of PD-1 with PD-L1 and
prevent the inactivation of the cytotoxic tel lymphocytes.
So to maximize the benefit of this immune checkpoint inhibitor, we have to select cases
carefully.
So how do we select cases?
The response to PD-1, PD-L1 depends upon tumor intrinsic factors as well as upon tumor
extrinsic factors.
So these are some of the factors which determine the response to immune checkpoint inhibitor
and of them the most robust one are PD-L1, MSI and tumor mutation burden.
Anti-PD-1 or PD-L1 antibodies are used in all stages of lung cancer and PD-L1 testing
is done in all stages of lung cancer.
And in all therapeutic contexts except knee-videgment where anti-PD-1 antibody is used without PD-L1
testing.
The PD-L1 testing is done through IHC, the multiple antibody clones available with different
testing platform and they have been marketed as complete SS either as a companion

diagnostic
or complementary diagnostics.
Some of these yield comparable results, for example 22C3, 28.8 and 263 but others are
either over expressed or under expressed.
Further the determination of the positive and negative PD-L1 expression vary depending
upon the organ site and the assay used and thus variation has been disconcerting both
for the oncologist and the pathologist.
So you can see several anti-PD-PD-1 clones, different types of interpretation, different
platforms, different antibodies and to streamline in the April of 2024 CAP, ESCO and MAMP they
decided that we can instead of using these assays we can use a lab develop test.
But these lab develop test must be adequately validated and this is a strong recommendation.
You can also use trial proven clone and platform but that is a conditional recommendation.
So the preference is for a lab develop test and not for the trial proven test because
that acts as an impediment to the greater utilization of PD-L1 testing.
And because we don't have any recommendation as to how we validate our test, so we use
CAP guidelines and for that we need to have 20 positive and 20 negative and the 20 positive
should be close to the threshold challenges that is 1% and 50%.
And the interpretation is that you score only two more cells.
You can score all cells with membrane sustaining.
The membrane sustaining can be strong, can be weak, learn to exclude necrophages and
just give the percentage of cells positive.
You don't have to do any other jiggery pokery, just 2PSC.
But despite all these harmonizations, intestines, interpretation, the PD-L1 is still an imperfect
biomarker.
It is an imperfect biomarker because those which have crossed the threshold amongst them
in the first line as well as in the subsequent line, the response rate is not better than
40%.
And those who have fallen below the threshold, even in them there is a response reaching
up to 15% in certain situations.
So the positive predictive value, the negative predictive value, both of the PD-L1 testing
is sub-optimal.
It's not the best.
So can we improve on the biomarkers in use of immunotherapy?
And as talked earlier, the MSI.
MSI is seen in 1% of the lung cancers, only 1%.
And 70% of these lung cancers also have over-rific PD-L1.
So you will get only 0.3% exclusive by doing MSI testing.
So if you will test 400 cases, you will get one additional exclusive MSI positive case,
which is not PD-L1.
So this is misallocation of resources.
You should not use it.
And the NCCN, ASCO, NSMO do not recommend MSI testing for immune checkpoint

inhibitors
in lung cancer.
Can I have some water?
And then we go to the tumor mutation, but which is basically number of somatic mutations,
including base substitution, insertion and deletion per megabase of tumor genome.
The biological significance is greater than mutation greater is the likelihood of having
a new antigen, which is immunogenic.
So basically, you're buying too many lottery tickets.
And initially, this was assess done whole exome sequencing.
And when it was assess done whole exome sequencing, there was a numerical cut off.
And this proved to be very useful, both with anti-CTL1 and NTPD1 use.
The responses really matched.
And then came this subsequently, using TARC.
I was given 15 minutes.
So I'll take 15 minutes.
Traditionally, it was traditionally assessed using whole exome sequencing.
But subsequently, the targeted NGS panels have been used, which were found to be comparable.
And a cut off of 10 mutations per megabase was given.
And that's where the story went sour.
The story went sour.
Because when in the real world, it was seen using this cut off, 25% for 26% patient showed
response when they were beyond the threshold.
And 5% to 10% is still showed response when they were below the threshold.
So the clinical utility has been questioned.
The doubts have been caused.
And this is what happened.
Several papers in 22 and 23 ran down the TMB.
So the TMB fell from the grace.
And with D.A. with D.U.T.M.B. as a biomarker for K.Truda in solid tumors in October 2023.
And in deference to these findings, the CAP and S.CO have also withdrawn TMB as a single
indicator for immune check point therapy.
It is not valid anymore.
So TMB is not valid on its own.
However, there is a rash tail.
It indicates that there is a load of new antigens, which can be recognized by immune
system.
But it is not taking into account the tumor microenvironment, which may not be favorable.
So if we combine both, can we make it a better predictor?
And that's what was proved in the post hoc analysis of CHACMATE 26, where high TMB and
high PDL1 were associated with higher objective response rate and a longer PFS compared to
the rest of the cohort.
And this was further proven in a very large trial from Dana-Fabber and MSKCC 1577 patient.
And if you combine intermediate PDL1 expression and high PDL1 expression with the high tumor
mutation burden, then you see the objective response as rows from 18.7 to 58 to 58 to
56.
The PFS improved from 2.6 to 30.6.

Drastic improvement happened when these two were combined.
So there is a case of using the synergy of a PDL1 with TMB to improve the predictive
power of this biomarker.
And then we can also integrate TMB with gene expression profile.
We can look whether the mutant carrying sequences are getting expressed or not expressed.
Are getting transcribed or not?
If they're not getting transcribed, there will be no protein.
So there will be no new antigen.
So to really know that the new antigen cell for me, they must get expressed.
So we see them.
And we also see the expression of the microenvironment, where that microenvironment is tumor inflammatory
effector cell promoter or inhibitor.
So we can use the TMB with gene expression profiling.
And we will also enhance the predictive power of the tumor mutation burden, how, by looking
only at the index.
It was recognized long back that in results cell carcinoma, TMB is low.
Then they went on to find the tumor indel burden.
Intubor indel burden was low, but yet patients were responding.
So what was the cause?
It was recognized that indel, especially out of frame, indel, frame, shift indel can produce
ninefold more new antigens.
And if you have that many more new antigens, you are likely to get some antigens which
are highly immunogenic and get a response.
And I will exhort you to read these two articles.
One is a commentary, one is a work.
And then we can also use the TMB data.
We can take the TMB data, we can take the VCF5, run it through the variant effect predictor
and then put it on this file.
This bioinformatic pipeline, PVACSAC or several others.
And when you run them on these bioinformatic pipeline, you can predict the peptide sequence
generated from tumor specific mutations.
You can define the matrix such as sequence, foreignness, mu antigen, clonality, epitope,
density.
You can also find out whether they have high MHC binding affinity and immunogenicity or
not.
And it can prioritize the new antigen and tell you that these are the ten mu antigens which
are highly immunogenic and you can use the immune check-pike therapy.
And then there are certain somatic mutations which have been shown to either enhance the
response or blunt the response.
So KRAS and TB53 to other can enhance the response in lung cancer, JAC3 because it promotes
the interferon gamma pathic and enhance, HLAB44 super-type, those who have this, they have
shown greater response to immune check point inhibitor and likewise.
So there are several papers, you can go through them.
And then there is a genomic signal which, genomic molecular score which has been developed

using air genes.
So based upon whether they are carrying a mutation or not carrying a mutation, the certain weightage
is allocated to them, a score is generated and it has been shown those who have a low
genomic mutation signature, they do inferior to those which have a high genomic mutation
signature, the blue lines, both in terms of PFS and OS.
And then beyond these current biomarkers, you also have several other biomarkers.
For example, you can look for interferon gamma signatures, you can look for effective T cells
and the picturesque of exhausted T cells in the circulation.
It has been shown if these two cells are present in high number, then this individual is more
likely to respond to anti-PD1, PDL1 therapy.
And then you can look at the other co-inhibitors.
You will look at several plasma biomarkers, which are basically related to these secondary
immune checkpoint and primary immune checkpoint and the double-standard DNA repair mechanism.
More and more emphasis is being given on them.
If there is a DNA repair damage pathway, the response to immune checkpoint inhibitor is
more likely especially CD-CAT-12.
And the bi-LA-gloss of CD-CAT-12 produces lots of internal tandem duplicates and mutations
which can then create new antigens.
And of course, the microbiome, the diversity of the microbiome and certain specific species
promote immune checkpoint inhibitor response while the others blunt it.
So the takeaways, PDL1 expression, standard of care, TMB trashed, we can salvage it by
combining it with something.
MSI is useless, don't do it.
And if MSI is 1%, that is what I was trying to tell with you.
MSI is 1%, this is somatic.
So Lynch, how much?
5% of that.
So 0.05%.
So she was actually chasing 0.05%.
And then advances in the new antigen prediction, very promising.
I think most promising aspect of this.
And many other biomarkers like co-occurring mutations got microbiota, a weaker but relevant
biomarker's in development.
And thank you for your patience and not yawning.