Thanks to CNST for asking me to talk about my research. In 15 minutes I’ll give you a whirlwind tour of what I did with my summer.
PURPOSE OF THIS TALK

- Make you want to do what I did:
  - Basic Science Research
  - Clinical Experience
  - Funding:
    - CNST
    - Kevin Heaney and Judi Marvel Charities Summer Brain Tumor Research Fellowships At CHOP
- Teach you something
BRAF is one of many proteins found in the MEK/ERK pathway that signals cell growth. BRAF is a kinase, which means it phosphorylates things. Phosphorylation is denoted by “-P”. Gain of function mutations anywhere in this pathway can obviously lead to cell growth and eventually a tumor.
BRAF mutations are commonly found in other tumors with a poor prognosis. V600E is a valine to glutamine mutation, which is found in ~7% of human malignancies and ~60% of melanomas. It is also the most frequent mutation in papillary thyroid cancer which is ~80-90% of thyroid cancers. Predicts outcomes in colorectal cancer. Found in 95% of high grade gliomas.

Fusion mutation found in 100% of low grade gliomas.

Plexxikon 4720 is one variation of a BRAF inhibitor that was specifically created to target the V600E mutation. There are other variations of this Plexxikon drug but this is the one we use. Ideally any time you can specifically target the oncogenic protein you could maximize positive effects and limit toxicity. Based on the positive effects of this drug on V600E containing tumors, researchers in my lab thought it may also work in fusion tumors, but it did not. This basic back story is the set up for my research.
Panel A: Patient with melanoma

Panel B: Same patient after treatment with PLX

Panel C: Same patient after melanoma recurrence on PLX

4 patients shown with melanoma before (first panel) and after (second panel) PLX treatment.

Ongoing research shows that melanoma patients with V600e mutations are responsive to PLX.

What our lab, and others like ours, is trying to prove is that PLX should be applied to other tumors with BRAF mutations. This makes sense because if BRAF is the oncogenic protein, specifically targeting it should be efficacious regardless of the organ that is affected.

The problems we have run into is that all BRAF mutations are not created equally. Tumors with V600e mutations are inhibited by PLX while others are activated. This is an obvious problem with brain tumors in which activation and growth could prove lethal very quickly.
So where do I come in?

Even though PLX is currently being tested in patients with BRAF mutations (mostly melanoma patients), there is no research demonstrating the effects of PLX on the actual BRAF protein. So my original research goal was to fill in this gap by isolating the mutated BRAF proteins and note the effects of PLX.

This should have been relatively easy, as isolating proteins is easy enough to do (or so I thought), and high yield as this was a gap in the literature. I soon found out why this isolated protein was absent from the literature, it proved impossible to isolate. After trying all lab techniques at our disposal, I suggested that we had to be missing a key component. After all, we were trying to isolate this protein from bacteria with no other human proteins (of course this ways the point-to isolate it). My PI mentioned that it had been suggested that BRAF was a HSP 90 substrate. This was truly an AHA moment. I did some research and discovered that wild-type (normal) BRAF is not a HSP 90 substrate, but it appears that mutated BRAF is.

We tested this theory by trying to isolate wild-type BRAF without HSP 90 which was successful. Then we took the mutated BRAF and added HSP 90 protein to the bacteria cells, and voila we isolated the protein! Mind you this hadn’t been done before.
You may be asking why does that make sense? If we remind ourselves of the function of HSP 90: it is a chaperone protein, which means it is a protein that helps other proteins assemble correctly. It sits open like a jar, you insert your protein that needs help, close the jar, shake and bake, then the jar opens and out pops your functional protein. In tumors that aren’t playing by the rules, it makes sense they would need help assembling their awkward mutated proteins in order for them to be functional.

That’s exciting but who cares?
This is exciting because we accidentally stumbled upon something potentially very important. It appears that these tumor cells need HSP 90 to assemble their mutated proteins. If that’s true, what would happen if we inhibited HSP 90 and took that help away. Fortunately there is a drug out there that does just that.

**Geldanamycin** is an antibiotic that binds to **Hsp90** (Heat Shock Protein 90) and inhibits its function. Geldanamycin is hepatotoxic which has led to the development of geldanamycin analogues, in particular analogues containing a derivatisation at the 17 position:17AAG.

17AAG has been in clinical trials since 1999. Now phase II trials against melanoma, breast, prostate, and thyroid cancer.
At this point we want to remind ourselves of BRAF’s job in this pathway. Remember BRAF is a KINASE. We can measure BRAF directly but we also want to know if it’s active because the protein levels we measure could either be working or non-functional and just hanging around, and this will be relevant when we look at some data. So the way we measure BRAF activity is to measure the next protein in the chain which is MEK. MEK could either by phosphorylated or unphosphorylated, and of course we care about phosphorylated MEK. And as you can see once MEK is phosphorylated it does its own thing as a part of signaling cell growth. So we can use P-MEK for 2 things: it is a great measure of BRAF activity and we can also use it as a surrogate for cell growth.
Before we start looking at data we just want to quickly understand how these drugs were added. In many of these experiments we used 2 drugs, with 3 concentrations each. We laid out the combined concentrations by using a matrix like this. As you can see we end up with 9 different combinations ranging from no drug at all to the highest concentration of both drugs. These numbers demonstrate how the data appears when put on a straight line so that one drug varies (GM) while the other stays constant, then we increase the dosage of the second drug (PLX) and vary the GM within that.

These are uM drug concentrations

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First we want to talk about the experiments on the fusion mutations because there is more data for the V600e which we’ll discuss next.
These experiments were performed in lab created tumor cells.

We took regular fibroblasts and infected them with mutated BRAF so they would behave like tumor cells. Then we added 2 drugs to them in the manner I described, so looking at this graph on the left of each graph we have no drug and on the right we have the highest drug concentrations.

There are several fusion mutations, but these graphs represent how all of them behaved. The way we get this information is after we add the drugs, the next day we split the cells open and use a series of techniques to measure the proteins present. And as I mentioned we looked at 2 different proteins, BRAF (obviously) and P-MEK to measure activity.

The main take away from these graphs is illustrated by the step-down yellow line, which shows that increasing concentrations of GM reduces not only BRAF protein expression, but also P-MEK (which is what we really care about since it measures BRAF activity).

These mutations were trouble before because, as I mentioned earlier, the BRAF inhibitor PLX actually causes some activation with higher concentrations which you can see if you notice the 1st and 4th bars on each graph.
Seizing on the information from the previous experiment we wanted a better idea of how GM affects actual cell growth. So this is a different type of experiment where we only used one drug (GM) and allowed the cells to grow for one week (which is more along the lines of what would happen with actual treatment). And instead of counting proteins we counted DNA to determine how many cells were present. And as you can see adding GM has a drastic dose dependent affect on cell growth.

This is exciting because this isn’t just protein expression it’s actual cell growth, which is what matters when these cells are located in your brain. These numbers represent percentages so you can see at the highest concentrations the drug reduced cell growth by over 90%.
Ok moving on to the V600e mutations
This is another experiment which was done in lab created cells. So these started out as normal cells and we gave them a mutation that made them behave like tumor cells. In this experiment we used 2 drugs similar to the way we did before, and after we split the cells open we looked at the same 2 proteins.

As you can see these results are different because instead of only 1 drug having an effect, both drugs have an effect so that if you look at the PMEK graph the drugs work together to give you the most significant effect when the drugs are combined. This is what you actually want to see from drugs that are used in therapy because in most cases tumors are very good at finding ways to get around one treatment. So when you have multiple drugs that work in different ways you increase the likelihood that the effect will last.

Also interesting about these 2 graphs is that they don’t have the exact same shape. You can see that the BRAF concentrations actually go up, but as I mentioned that’s why we use the PMEK to compare because now we know that higher amounts of BRAF isn’t actually functioning at a higher level.
Fortunately we have real tumor cells taken from patients with brain tumors that contained V600E mutations, so we can compare our lab data to real life tumors. These images came from a growth experiment like the one I mentioned early. The entire image isn’t in focus because we allowed the cells to grow in 3 dimensions, which is also closer to real life.

Just looking at these images we can see a difference between the cells that received no drug and the cells that received drug. These boxes represent the response when the drug was added alone versus when it was combined, and these are pictures from the highest concentration so you are seeing the maximal effect. Notice the PLX box looks better than no drug, but the GM and combination box might look a little better. By better I mean the cells are small and “quiescent” looking compared to what is clearly active cell growth happening without the drug.

This is only one of the tumors.
This is another brain tumor that went through the same experiment. And interestingly you can see that the there is the same type of pattern of improvement moving from no drug to combination, but notice that the cell growth pattern looks different. These cells tend to spread outward more rather than spherically. The shape of the cell growth is irrelevant, it’s just an interesting aside.
And the is the 3rd and final tumor type. I like these images because they show that the drugs are really having an effect on the how the cells behave. But we also have data for these experiments.
This is the data from the first image that I showed you. Notice on the left is the PMEK data from the lab created cells, which I mentioned we try to use as a surrogate of cell growth. This is compared to actual cell growth that we can measure. And what you see is noted by the orange arrow that this tumor actually behaves mostly like what we would expect in that both drugs have an effect and work together.
This is the data from the second image I showed you and similar to the first tumor, we see the tumor behaving somewhat like the lab data though definitely less so than the first tumor.
This is the data from the third image I showed you. What is really interesting here is that PLX does have an effect if you look at the pink step down line. But what is really interesting is that the GM has a drastic effect noted by the orange arrow.

So why does this matter well...
The differences in this growth data matter because all of these tumors have the same V600E mutation, but they don’t all behave the same. And what this tells us is that even though they all have identical BRAF mutations (which we think is most important), there is clearly something going on that would make these tumors respond differently to treatment. Since these are real tumor cells they have many other mutations. By figuring out other proteins involved in this oncogenic process we may be able to pin point what makes some tumors more easily treatable than others, and also what are the differences that will allow some tumors to get around treatment.
So why did I start this out by calling it a cinderella story? Well what’s listed up there is very important. GM is safe, cheap, FDA approved, formulated for human oral intake, and can be translated into treatment very quickly.

Most resources in the drug industry are being directed at drugs like PLX which targets specifically the mutated protein, which seems ideal. While this is the type of drug that many people have started to move away from because it is not specific at all, it could potentially target all cells that rely on HSP90 (nearly every cell). And most cells use it responsibly to make normal healthy proteins. So to a drug company this drug looks like a bad toxin. But what we’re hoping is that in reality, tumors rely much more heavily on HSP 90 than other cells so that this drug could disproportionately disrupt tumor activity rather than normal cell activity. While it may not turn out the be the primary drug, this could be a very strong adjuvant to those other specific drug therapies.
RESEARCH CONCLUSIONS

- HSP90 is important helper of mutated BRAF
- Targeting HSP90 reduces tumor growth
- HSP90 inhibitor may be novel therapy for fusion mutations
  - PLX causes activation—not helpful
- HSP90 inhibitor may be useful combination therapy with PLX for tumors with V600e mutations
  - Multiple therapies reduce chance for evolution
I enjoyed this experience because it gave me a taste of what real science is like. In reality we ended up with far more questions than answers, which I will most likely continue to explore in a year out experience.

Some of those questions are:

- Few answers, many questions:
  - What is MOA of HSP90 in tumor cells?
    - Which proteins does it target?
  - Why don’t all V600e mutations respond predictably?
    - Other MAPK/ERK pathway mutations?
  - How do these drugs act in living organism?
    - HSP90 is omnipresent
    - Mice
What I really hope you gained from this:
Basic science (not just clinical) can be translational too
THANKS

- Dr. Resnick
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- Resnick-Storm Lab Team
- CNST