WEBVTT

NOTE duration:"00:50:24.8000000"

NOTE language:en-us

NOTE Confidence: 0.86679626

00:00:16.910 --> 00:00:18.786 Alright guys, why don't we go ahead

NOTE Confidence: 0.86679626

00:00:18.790 --> 00:00:20.674 and get started so we're having a

NOTE Confidence: 0.86679626

00:00:20.674 --> 00:00:22.560 zoom webinar format for this talk so

NOTE Confidence: 0.86679626

00:00:22.560 --> 00:00:24.314 Doctor Sakamoto will give her talk and

NOTE Confidence: 0.86679626

00:00:24.314 --> 00:00:26.330 then at the end we'll have questions.

NOTE Confidence: 0.86679626

00:00:26.330 --> 00:00:28.207 I think you're going to have to

NOTE Confidence: 0.86679626

00:00:28.207 --> 00:00:29.821 put your questions either in the

NOTE Confidence: 0.86679626

00:00:29.821 --> 00:00:32.238 Q&A or in the chat and then I can

NOTE Confidence: 0.86679626

00:00:32.240 --> 00:00:34.160 read them to her just given

NOTE Confidence: 0.86679626

00:00:34.160 --> 00:00:35.860 the format of this talk.

NOTE Confidence: 0.86679626

00:00:35.860 --> 00:00:37.318 So I'm Jeannie Hendrickson.

NOTE Confidence: 0.86679626

00:00:37.318 --> 00:00:38.410 I'm representing the

NOTE Confidence: 0.86679626

00:00:38.410 --> 00:00:39.499 Yellow Cooperative Center

NOTE Confidence: 0.83495444

00:00:39.500 --> 00:00:40.952 of Excellence in hematology

NOTE Confidence: 0.83495444

00:00:40.952 --> 00:00:42.404 for the enrichment program,

NOTE Confidence: 0.83495444

00:00:42.410 --> 00:00:44.595 and we're extremely happy to have

NOTE Confidence: 0.83495444

00:00:44.595 --> 00:00:46.783 Doctor Sakamoto who's giving a talk,

NOTE Confidence: 0.83495444

00:00:46.783 --> 00:00:50.053 a talk to us all the way from Stanford.

NOTE Confidence: 0.83495444

00:00:50.053 --> 00:00:51.144 Virtually, of course,

NOTE Confidence: 0.83495444

00:00:51.144 --> 00:00:52.964 she's a professor in pediatric

NOTE Confidence: 0.83495444

00:00:52.964 --> 00:00:54.420 hematology oncology at Stanford,

NOTE Confidence: 0.83495444

00:00:54.420 --> 00:00:56.246 and her research involves signaling

NOTE Confidence: 0.83495444

00:00:56.246 --> 00:00:58.062 pathways and gene regulation in

NOTE Confidence: 0.83495444

00:00:58.062 --> 00:00:59.514 normal and aberrant masterpieces,

NOTE Confidence: 0.83495444

00:00:59.520 --> 00:01:00.975 including bone marrow failure.

NOTE Confidence: 0.83495444

00:01:00.975 --> 00:01:04.255 And today she's going to focus her talk on

NOTE Confidence: 0.83495444

00:01:04.255 --> 00:01:07.190 this as it relates to Diamond Black fan.

NOTE Confidence: 0.83495444

00:01:07.190 --> 00:01:09.145 Nina, so I will turn it over

NOTE Confidence: 0.83495444

00:01:09.145 --> 00:01:11.005 to her and again thank you so

NOTE Confidence: 0.83495444

00:01:11.005 --> 00:01:12.330 much for joining us today.

NOTE Confidence: 0.8300267

00:01:13.800 --> 00:01:15.270 Thank you so much Gene.

NOTE Confidence: 0.8300267

00:01:15.270 --> 00:01:16.730 I really appreciate the invitation.

NOTE Confidence: 0.8300267

00:01:16.730 --> 00:01:19.226 I also want to thank Pat and Diane

NOTE Confidence: 0.8300267

00:01:19.226 --> 00:01:21.087 especially for this kind of rotation

NOTE Confidence: 0.8300267

00:01:21.087 --> 00:01:23.691 and what I'm going to do today for the

NOTE Confidence: 0.8300267

00:01:23.691 --> 00:01:26.114 next 45 minutes or so is to talk about

NOTE Confidence: 0.8300267

00:01:26.114 --> 00:01:28.193 some of the recent work that we've

NOTE Confidence: 0.8300267

00:01:28.193 --> 00:01:30.454 been doing on signaling pathways in

NOTE Confidence: 0.8300267

00:01:30.454 --> 00:01:32.660 the pathogenesis and treatment of TBI.

NOTE Confidence: 0.8300267

00:01:32.660 --> 00:01:34.748 So just to say I have no good disclosure,

NOTE Confidence: 0.8300267

00:01:34.750 --> 00:01:36.470 I have no disclosures that

NOTE Confidence: 0.8300267

00:01:36.470 --> 00:01:38.190 are related to this work.

NOTE Confidence: 0.8300267

00:01:38.190 --> 00:01:40.297 So Diamond life and anemia is a

NOTE Confidence: 0.8300267

00:01:40.297 --> 00:01:42.339 very rare boomer failure syndrome,

NOTE Confidence: 0.8300267

00:01:42.340 --> 00:01:45.252 and I'll discuss some of the background

NOTE Confidence: 0.8300267

00:01:45.252 --> 00:01:47.669 and clinical features of this disease.

NOTE Confidence: 0.8300267

00:01:47.670 --> 00:01:50.274 Also, the role of an email like

NOTE Confidence: 0.8300267

00:01:50.274 --> 00:01:53.268 high knees in DBA models and then

NOTE Confidence: 0.8300267

00:01:53.268 --> 00:01:55.950 targeting and OK for the treatment

NOTE Confidence: 0.8300267

00:01:56.035 --> 00:01:59.095 of DBA and the last part of the talk.

NOTE Confidence: 0.8300267

00:01:59.100 --> 00:02:02.214 I will talk about a new project on 71

NOTE Confidence: 0.8300267

00:02:02.214 --> 00:02:05.758 in erythropoiesis and EPA pathogenesis.

NOTE Confidence: 0.8300267

00:02:05.760 --> 00:02:08.168 So DPA is a very rare congenital

NOTE Confidence: 0.8300267

00:02:08.168 --> 00:02:10.378 bone marrow failure syndrome that's

NOTE Confidence: 0.8300267

00:02:10.378 --> 00:02:12.586 associated with macrocytic anemia,

NOTE Confidence: 0.8300267

00:02:12.590 --> 00:02:15.146 congenital defects, Anna risk of cancer.

NOTE Confidence: 0.8300267

00:02:15.150 --> 00:02:17.712 This disease is generally diagnosed in

NOTE Confidence: 0.8300267

00:02:17.712 --> 00:02:21.126 early childhood, less than a year of age.

NOTE Confidence: 0.8300267

00:02:21.130 --> 00:02:24.441 The incidence is seven in a million

NOTE Confidence: 0.8300267

00:02:24.441 --> 00:02:27.473 and approximately 20 to 40 new cases

NOTE Confidence: 0.8300267

00:02:27.473 --> 00:02:30.100 are diagnosed per year in the US,

NOTE Confidence: 0.8300267

00:02:30.100 --> 00:02:30.950 in Canada.

NOTE Confidence: 0.76300555

00:02:34.190 --> 00:02:35.879 Download iPad anemia.

NOTE Confidence: 0.76300555

00:02:35.879 --> 00:02:39.257 Clinical features are quite diverse and

NOTE Confidence: 0.76300555

00:02:39.257 --> 00:02:42.392 there are many patients who present

NOTE Confidence: 0.76300555

00:02:42.392 --> 00:02:45.525 with short stature shown here and

NOTE Confidence: 0.76300555

00:02:45.525 --> 00:02:48.340 these Twins upper limb abnormalities

NOTE Confidence: 0.76300555

00:02:48.340 --> 00:02:51.734 including thumb and facial and pallet

NOTE Confidence: 0.76300555

00:02:51.734 --> 00:02:55.136 malformations which can occur with certain

NOTE Confidence: 0.76300555

00:02:55.136 --> 00:02:57.790 ribosomal protein subunit mutations.

NOTE Confidence: 0.76300555

00:02:57.790 --> 00:03:00.358 Patients can present with small eyes,

NOTE Confidence: 0.76300555

00:03:00.360 --> 00:03:03.356 kidney defects, but only for 30% of

NOTE Confidence: 0.76300555

00:03:03.356 --> 00:03:05.486 all patients have physical findings,

NOTE Confidence: 0.76300555

00:03:05.490 --> 00:03:07.505 which makes it very challenging

NOTE Confidence: 0.76300555

00:03:07.505 --> 00:03:10.943 when you have a patient who may not

NOTE Confidence: 0.76300555

00:03:10.943 --> 00:03:13.178 manifest significant anemia and may

NOTE Confidence: 0.76300555

00:03:13.178 --> 00:03:15.767 be identified by their adult intern,

NOTE Confidence: 0.76300555

00:03:15.770 --> 00:03:16.582 internist, physician,

NOTE Confidence: 0.76300555

00:03:16.582 --> 00:03:19.018 who notices that they have microcytosis

NOTE Confidence: 0.76300555

00:03:19.018 --> 00:03:21.329 but no other physical features,

NOTE Confidence: 0.76300555

00:03:21.330 --> 00:03:25.098 and we've had a few of these patients

NOTE Confidence: 0.76300555

00:03:25.098 --> 00:03:28.879 come to clinic in our at Stanford.

NOTE Confidence: 0.76300555

00:03:28.880 --> 00:03:31.165 The treatment for Diamondback anemia

NOTE Confidence: 0.76300555

00:03:31.165 --> 00:03:33.456 is typically steroids, chronic ritzel,

NOTE Confidence: 0.76300555

00:03:33.456 --> 00:03:35.746 transfusions and stem cell transplantation.

NOTE Confidence: 0.76300555

00:03:35.750 --> 00:03:38.498 For patients who are steroid refractory

NOTE Confidence: 0.76300555

00:03:38.498 --> 00:03:40.330 or chronically transfusion dependent,

NOTE Confidence: 0.76300555

00:03:40.330 --> 00:03:42.615 and these are all associated

NOTE Confidence: 0.76300555

00:03:42.615 --> 00:03:43.986 with significant morbidities,

NOTE Confidence: 0.76300555

00:03:43.990 --> 00:03:44.906 including immunosuppression,

NOTE Confidence: 0.76300555

00:03:44.906 --> 00:03:47.654 iron overload, graph versus host disease,

NOTE Confidence: 0.76300555

00:03:47.660 --> 00:03:50.402 there are newer therapies that have

NOTE Confidence: 0.76300555

00:03:50.402 --> 00:03:51.773 come been proposed.

NOTE Confidence: 0.76300555

00:03:51.780 --> 00:03:54.986 For example, L, leucine, the amino acid.

NOTE Confidence: 0.76300555

00:03:54.990 --> 00:03:57.445 Many of you are familiar

NOTE Confidence: 0.76300555

00:03:57.445 --> 00:03:59.409 with the clinical trial.

NOTE Confidence: 0.76300555

00:03:59.410 --> 00:04:02.056 It's been directed by Jeff Lipton,

NOTE Confidence: 0.76300555

00:04:02.060 --> 00:04:03.383 an Adreno blocos.

NOTE Confidence: 0.76300555

00:04:03.383 --> 00:04:05.147 They showed a modest

NOTE Confidence: 0.76300555

00:04:05.147 --> 00:04:06.470 improvement with leucine,

NOTE Confidence: 0.76300555

00:04:06.470 --> 00:04:08.234 although the doses were

NOTE Confidence: 0.76300555

00:04:08.234 --> 00:04:10.439 much lower and for safety,

NOTE Confidence: 0.76300555

00:04:10.440 --> 00:04:12.756 but there were some patients who

NOTE Confidence: 0.76300555

00:04:12.756 --> 00:04:14.300 did experience improvement in

NOTE Confidence: 0.76300555

00:04:14.364 --> 00:04:16.170 the transfusion requirements.

NOTE Confidence: 0.76300555

00:04:16.170 --> 00:04:18.696 So Tatter septis Eligant wrapped that

NOTE Confidence: 0.76300555

00:04:18.696 --> 00:04:21.359 inhibits the TGF beta signaling pathways

NOTE Confidence: 0.76300555

00:04:21.359 --> 00:04:24.059 that also has been shown recently

NOTE Confidence: 0.76300555

00:04:24.059 --> 00:04:26.750 to improve with police in patients,

NOTE Confidence: 0.76300555

00:04:26.750 --> 00:04:30.038 for example with Milo dysplastic syndrome.

NOTE Confidence: 0.76300555

00:04:30.040 --> 00:04:33.190 But most of them are being

NOTE Confidence: 0.76300555

00:04:33.190 --> 00:04:34.240 cronian investigated.

NOTE Confidence: 0.7915295

00:04:36.550 --> 00:04:38.986 Why does summer deficiency in bone

NOTE Confidence: 0.7915295

00:04:38.986 --> 00:04:41.049 marrow failure syndrome is become

NOTE Confidence: 0.7915295

00:04:41.049 --> 00:04:43.281 more and more common in diseases

NOTE Confidence: 0.7915295

00:04:43.281 --> 00:04:45.441 such as Shockman Diamond syndrome

NOTE Confidence: 0.7915295

00:04:45.441 --> 00:04:47.866 which is another congenital bone

NOTE Confidence: 0.7915295

00:04:47.866 --> 00:04:50.180 marrow failure syndrome with other

NOTE Confidence: 0.7915295

00:04:50.180 --> 00:04:52.305 physical findings, deletion 5 Q.

NOTE Confidence: 0.7915295

00:04:52.310 --> 00:04:54.234 Milo dysplastic syndromes which

NOTE Confidence: 0.7915295

00:04:54.234 --> 00:04:56.639 is associated with our PS14

NOTE Confidence: 0.7915295

00:04:56.639 --> 00:04:58.279 haploinsufficiency that's been described

NOTE Confidence: 0.7915295

00:04:58.279 --> 00:05:00.827 by Ben Ebras group several years ago.

NOTE Confidence: 0.7915295

00:05:00.830 --> 00:05:02.790 These are all considered diseases

NOTE Confidence: 0.7915295

00:05:02.790 --> 00:05:04.750 that are ribosome opathy's with

NOTE Confidence: 0.7915295

00:05:04.811 --> 00:05:06.620 defective ribosome Biogenesis.

NOTE Confidence: 0.7915295

00:05:06.620 --> 00:05:08.460 And function.

NOTE Confidence: 0.7915295

00:05:08.460 --> 00:05:11.166 Over 80% of DBA patients have

NOTE Confidence: 0.7915295

00:05:11.166 --> 00:05:12.970 mutations in ribosomal protein.

NOTE Confidence: 0.7915295

00:05:12.970 --> 00:05:15.676 Some units resulting in ribosome dysfunction,

NOTE Confidence: 0.7915295

00:05:15.680 --> 00:05:18.968 an impaired protein translation.

NOTE Confidence: 0.7915295

00:05:18.970 --> 00:05:21.514 This is just a pie chart that shows

NOTE Confidence: 0.7915295

00:05:21.514 --> 00:05:23.764 the various percentages of patients

NOTE Confidence: 0.7915295

00:05:23.764 --> 00:05:25.816 who have ribosomal mutations.

NOTE Confidence: 0.7915295

00:05:25.820 --> 00:05:28.550 You can see that among the most

NOTE Confidence: 0.7915295

00:05:28.550 --> 00:05:30.820 common is RPS 1925% RP 11.

NOTE Confidence: 0.7915295

00:05:30.820 --> 00:05:33.762 About 5% in RP 11 and five have been

NOTE Confidence: 0.7915295

00:05:33.762 --> 00:05:36.697 associated more with craniofacial defects,

NOTE Confidence: 0.7915295

00:05:36.700 --> 00:05:38.785 and there are several other

NOTE Confidence: 0.7915295

00:05:38.785 --> 00:05:40.870 mutations have been identified but

NOTE Confidence: 0.7915295

00:05:40.938 --> 00:05:42.716 30% have non ribosomal mutations.

NOTE Confidence: 0.7915295

00:05:42.716 --> 00:05:45.560 In an example is what Vijay Sankar

NOTE Confidence: 0.7915295

00:05:45.560 --> 00:05:47.580 had reported that God would.

NOTE Confidence: 0.7915295

00:05:47.580 --> 00:05:49.368 Mutations can also contribute

NOTE Confidence: 0.7915295

00:05:49.368 --> 00:05:50.709 to this disease.

NOTE Confidence: 0.7915295

00:05:50.710 --> 00:05:52.024 But there are several more mutations

NOTE Confidence: 0.7915295

00:05:52.024 --> 00:05:53.529 that have yet to be identified.

NOTE Confidence: 0.77043605

00:05:55.650 --> 00:05:57.942 This is a schematic that basically

NOTE Confidence: 0.77043605

00:05:57.942 --> 00:06:00.329 summarizes some of the defects that

NOTE Confidence: 0.77043605

00:06:00.329 --> 00:06:02.699 have been identified in the ribosomes

NOTE Confidence: 0.77043605

00:06:02.699 --> 00:06:05.226 are made synthesis pathway primarily

NOTE Confidence: 0.77043605

00:06:05.226 --> 00:06:07.887 the Treacher Collins syndrome, which,

NOTE Confidence: 0.77043605

00:06:07.887 --> 00:06:09.635 Interestingly does not usually

NOTE Confidence: 0.77043605

00:06:09.635 --> 00:06:11.820 manifest with bone marrow findings.

NOTE Confidence: 0.77043605

00:06:11.820 --> 00:06:14.150 We have just keratosis congenita

NOTE Confidence: 0.77043605

00:06:14.150 --> 00:06:16.480 which most people are familiar

NOTE Confidence: 0.77043605

00:06:16.558 --> 00:06:18.820 with that can cause fibrosis as

NOTE Confidence: 0.77043605

00:06:18.820 --> 00:06:21.000 well as bone marrow failure.

NOTE Confidence: 0.77043605

00:06:21.000 --> 00:06:23.215 Cartilage hair hypoplasia has been

NOTE Confidence: 0.77043605

00:06:23.215 --> 00:06:24.987 associated with macrocytic anemia.

NOTE Confidence: 0.77043605

00:06:24.990 --> 00:06:26.770 And then of course, TBA,

NOTE Confidence: 0.77043605

00:06:26.770 --> 00:06:29.080 which can involve the small subunit

NOTE Confidence: 0.77043605

00:06:29.080 --> 00:06:31.400 or large subunit 60 S or 40th,

NOTE Confidence: 0.77043605

00:06:31.400 --> 00:06:33.180 and then shockman diamond syndrome.

NOTE Confidence: 0.7897504

00:06:35.860 --> 00:06:37.965 It's still very interesting that

NOTE Confidence: 0.7897504

00:06:37.965 --> 00:06:40.076 this disease, which is germline

NOTE Confidence: 0.7897504

00:06:40.076 --> 00:06:41.764 mutation of these Robertson,

NOTE Confidence: 0.7897504

00:06:41.770 --> 00:06:44.400 will proteins in other mutations

NOTE Confidence: 0.7897504

00:06:44.400 --> 00:06:46.504 involved primarily the erythroid

NOTE Confidence: 0.7897504

00:06:46.504 --> 00:06:49.366 lineages and what I thought is in

NOTE Confidence: 0.7897504

00:06:49.366 --> 00:06:51.641 this very simplified version of Rip

NOTE Confidence: 0.7897504

00:06:51.641 --> 00:06:53.729 Oasis is that the cells increase

NOTE Confidence: 0.7897504

00:06:53.729 --> 00:06:55.918 in number as they become Earth,

NOTE Confidence: 0.7897504

00:06:55.918 --> 00:06:58.724 will glass and somehow this results in

NOTE Confidence: 0.7897504

00:06:58.724 --> 00:07:00.760 increased requirement for ribosomes,

NOTE Confidence: 0.7897504

00:07:00.760 --> 00:07:02.870 most likely due to increase.

NOTE Confidence: 0.7897504

00:07:02.870 --> 00:07:04.980 Protein translation is required for

NOTE Confidence: 0.7897504

00:07:04.980 --> 00:07:06.668 cell proliferation and differentiation.

NOTE Confidence: 0.6911545

00:07:09.430 --> 00:07:10.980 The normal hematopoietic treated Mary.

NOTE Confidence: 0.6911545

00:07:10.980 --> 00:07:12.840 Are you familiar with already from?

NOTE Confidence: 0.6911545

00:07:12.840 --> 00:07:14.604 He might have put stem cells to

NOTE Confidence: 0.6911545

00:07:14.604 --> 00:07:16.545 come in my local gender Mega

NOTE Confidence: 0.6911545

00:07:16.545 --> 00:07:18.420 Carey Service Rd Ripper genders,

NOTE Confidence: 0.6911545

00:07:18.420 --> 00:07:19.970 and then the birth funding

NOTE Confidence: 0.6911545

00:07:19.970 --> 00:07:21.210 isn't calling from Eunice.

NOTE Confidence: 0.6911545

00:07:21.210 --> 00:07:24.010 Very earlier it'll bus stage all lead to

NOTE Confidence: 0.6911545

00:07:24.010 --> 00:07:26.557 eventually the maturing of red blood cells.

NOTE Confidence: 0.6911545

00:07:26.560 --> 00:07:29.620 In DBA, these very subunit mutations

NOTE Confidence: 0.6911545

00:07:29.620 --> 00:07:32.086 that have been described seemed

NOTE Confidence: 0.6911545

00:07:32.086 --> 00:07:34.822 to be a result in a block in

NOTE Confidence: 0.6911545

00:07:34.822 --> 00:07:37.428 early committed with blaster be.

NOTE Confidence: 0.6911545

00:07:37.430 --> 00:07:40.148 If you be a few istage,

NOTE Confidence: 0.6911545

00:07:40.150 --> 00:07:42.170 mostly resulting from haploinsufficiency

NOTE Confidence: 0.6911545

00:07:42.170 --> 00:07:45.200 inducing mutation and we know that

NOTE Confidence: 0.6911545

00:07:45.276 --> 00:07:46.812 homozygous mutations result in

NOTE Confidence: 0.6911545

00:07:46.812 --> 00:07:49.660 mouse models as well as in humans.

NOTE Confidence: 0.6911545

00:07:49.660 --> 00:07:51.524 Embryonic lethality so helpful

NOTE Confidence: 0.6911545

00:07:51.524 --> 00:07:52.922 insufficiency or heterozygous

NOTE Confidence: 0.6911545

00:07:52.922 --> 00:07:55.100 mutations are typically what results.

NOTE Confidence: 0.6911545

00:07:55.100 --> 00:07:57.710 What results in this disease.

NOTE Confidence: 0.6911545

00:07:57.710 --> 00:08:00.518 Ultimately leading to anemia.

NOTE Confidence: 0.6911545

00:08:00.520 --> 00:08:03.130 So we started out looking at

NOTE Confidence: 0.6911545

00:08:03.130 --> 00:08:04.870 variety of signaling molecules,

NOTE Confidence: 0.6911545

00:08:04.870 --> 00:08:07.606 one that was of interest to us initially

NOTE Confidence: 0.6911545

00:08:07.606 --> 00:08:09.883 was a transcription factor MYB and

NOTE Confidence: 0.6911545

00:08:09.883 --> 00:08:13.550 we know that this is a very important

NOTE Confidence: 0.6911545

00:08:13.550 --> 00:08:16.178 protein that regulates erythropoiesis.

NOTE Confidence: 0.6911545

00:08:16.180 --> 00:08:19.225 It's also associated with my little keemia.

NOTE Confidence: 0.6911545

00:08:19.230 --> 00:08:21.600 When Aberrantly expressed and it's been

NOTE Confidence: 0.6911545

00:08:21.600 --> 00:08:24.695 reported that one of the kinases that

NOTE Confidence: 0.6911545

00:08:24.695 --> 00:08:26.615 activates or phosphorylates Mibiz,

NOTE Confidence: 0.6911545

00:08:26.620 --> 00:08:27.600 NIMAL, Iconis,

NOTE Confidence: 0.6911545

00:08:27.600 --> 00:08:30.050 and this kinese is Assyrian

NOTE Confidence: 0.6911545

00:08:30.050 --> 00:08:31.030 threatening kinese.

NOTE Confidence: 0.6911545

00:08:31.030 --> 00:08:34.096 That is a revolutionary and evolutionary

NOTE Confidence: 0.6911545

00:08:34.096 --> 00:08:36.140 conserved margin activated protein

NOTE Confidence: 0.6911545

00:08:36.211 --> 00:08:38.864 kinase in the map kinase family member.

NOTE Confidence: 0.6911545

00:08:38.870 --> 00:08:41.026 Originally described when mutated

NOTE Confidence: 0.6911545

00:08:41.026 --> 00:08:43.721 result results in I development

NOTE Confidence: 0.6911545

00:08:43.721 --> 00:08:46.448 defects in just saffola is highly

NOTE Confidence: 0.6911545

00:08:46.448 --> 00:08:48.553 expressed in neural tissues and

NOTE Confidence: 0.6911545

00:08:48.632 --> 00:08:51.880 plays a critical role in a number of

NOTE Confidence: 0.6911545

00:08:51.880 --> 00:08:53.978 important cellular functions through

NOTE Confidence: 0.6911545

00:08:53.978 --> 00:08:57.173 the regulation of various transcription

NOTE Confidence: 0.6911545

00:08:57.173 --> 00:08:59.699 transcription factors such as nib.

NOTE Confidence: 0.6911545

00:08:59.700 --> 00:09:01.700 An OK also regulate signaling

NOTE Confidence: 0.6911545

00:09:01.700 --> 00:09:03.700 pathways involving wind beta catenin

NOTE Confidence: 0.6911545

00:09:03.763 --> 00:09:05.395 active in aisle 6 an notch,

NOTE Confidence: 0.6911545

00:09:05.400 --> 00:09:08.365 which are all very critical

NOTE Confidence: 0.6911545

00:09:08.365 --> 00:09:10.144 for normal hematopoiesis.

NOTE Confidence: 0.6911545

00:09:10.150 --> 00:09:12.518 So this is work done by Mark Walsh,

NOTE Confidence: 0.6911545

00:09:12.520 --> 00:09:13.995 who is a former postdoctoral

NOTE Confidence: 0.6911545

00:09:13.995 --> 00:09:15.175 fellow in my lab,

NOTE Confidence: 0.6911545

00:09:15.180 --> 00:09:18.659 and now is an instructor in Pediatrics.

NOTE Confidence: 0.6911545

00:09:18.660 --> 00:09:21.412 We used a model that had been used

NOTE Confidence: 0.6911545

00:09:21.412 --> 00:09:24.313 prior in my lab where we would

NOTE Confidence: 0.6911545

00:09:24.313 --> 00:09:27.000 transduced human CD 34 positive cells,

NOTE Confidence: 0.6911545

00:09:27.000 --> 00:09:28.188 hematopoietic stem progenitor

NOTE Confidence: 0.6911545

00:09:28.188 --> 00:09:30.168 cells with small hairpin RNAs,

NOTE Confidence: 0.6911545

00:09:30.170 --> 00:09:34.463 then knocked down either RPS 19 or RPO 11.

NOTE Confidence: 0.6911545

00:09:34.470 --> 00:09:36.410 Making lentiviral constructs with

NOTE Confidence: 0.6911545

00:09:36.410 --> 00:09:39.320 transduced and then show that we

NOTE Confidence: 0.6911545

00:09:39.397 --> 00:09:41.861 could get help low or half the

NOTE Confidence: 0.6911545

00:09:41.861 --> 00:09:44.125 expression at the protein level by

NOTE Confidence: 0.6911545

00:09:44.125 --> 00:09:46.750 this Western blot or M RNA levels.

NOTE Confidence: 0.6911545

00:09:46.750 --> 00:09:49.470 And this is the model that we used.

NOTE Confidence: 0.6911545

00:09:49.470 --> 00:09:51.549 And when Mark looked at the expression

NOTE Confidence: 0.6911545

00:09:51.549 --> 00:09:53.950 of an OK throughout, he matter.

NOTE Confidence: 0.6911545

00:09:53.950 --> 00:09:56.170 Police is we didn't see any

NOTE Confidence: 0.6911545

00:09:56.170 --> 00:09:57.630 significant differences in our bar.

NOTE Confidence: 0.6911545

00:09:57.630 --> 00:10:00.955 RPS 19 RP 11 knock down now.

NOTE Confidence: 0.6911545

00:10:00.960 --> 00:10:01.810 In contrast,

NOTE Confidence: 0.6911545

00:10:01.810 --> 00:10:03.935 looking at phosphorylation or activation

NOTE Confidence: 0.6911545

00:10:03.935 --> 00:10:07.609 of an OK when Mark noticed was that

NOTE Confidence: 0.6911545

00:10:07.609 --> 00:10:09.469 taking three different substrates,

NOTE Confidence: 0.6911545

00:10:09.470 --> 00:10:12.606 either an OK itself this is auto

NOTE Confidence: 0.6911545

00:10:12.606 --> 00:10:14.497 phosphorylates, nib or wrapped,

NOTE Confidence: 0.6911545

00:10:14.497 --> 00:10:17.371 or that there is significant increase

NOTE Confidence: 0.6911545

00:10:17.371 --> 00:10:19.994 in the activation of this kinese

NOTE Confidence: 0.6911545

00:10:19.994 --> 00:10:23.006 in the 1st 10 days of hematopoiesis

NOTE Confidence: 0.6911545

00:10:23.006 --> 00:10:26.058 and so this we decided to focus

NOTE Confidence: 0.6911545

00:10:26.058 --> 00:10:29.496 on an OK and looking at mid target

NOTE Confidence: 0.6911545

00:10:29.496 --> 00:10:31.989 genes with RPS 19 lockdown.

NOTE Confidence: 0.6911545

00:10:31.990 --> 00:10:32.863 Again, HSBC is,

NOTE Confidence: 0.6911545

00:10:32.863 --> 00:10:34.318 we see that their expression

NOTE Confidence: 0.6911545

00:10:34.318 --> 00:10:36.677 of Alamo two in California were

NOTE Confidence: 0.6911545

00:10:36.677 --> 00:10:37.537 significantly decreased.

NOTE Confidence: 0.6911545

00:10:37.540 --> 00:10:37.887 However,

NOTE Confidence: 0.6911545

00:10:37.887 --> 00:10:40.663 with the expression or knockdown of an OK,

NOTE Confidence: 0.7975294

00:10:40.670 --> 00:10:43.183 we see that that we could partially

NOTE Confidence: 0.7975294

00:10:43.183 --> 00:10:45.519 rescue the expression of these two genes.

NOTE Confidence: 0.7975294

00:10:45.520 --> 00:10:46.908 Obviously there other Trump,

NOTE Confidence: 0.7975294

00:10:46.908 --> 00:10:48.990 you know the regulators of nib,

NOTE Confidence: 0.7975294

00:10:48.990 --> 00:10:52.113 so that makes a lot of sense to us.

NOTE Confidence: 0.7975294

00:10:52.120 --> 00:10:53.158 I'm sorry, Kathy,

NOTE Confidence: 0.7975294

00:10:53.160 --> 00:10:56.880 can you go back one slide?

NOTE Confidence: 0.7975294

00:10:56.880 --> 00:10:59.320 What is one more? I'm sorry.

NOTE Confidence: 0.7975294

00:10:59.320 --> 00:11:02.576 What are you showing on the PC MIB?

NOTE Confidence: 0.7975294

00:11:02.580 --> 00:11:04.610 One forward PC MIB an?

NOTE Confidence: 0.7975294

00:11:04.610 --> 00:11:07.058 What's the PC MIB? There is

NOTE Confidence: 0.71488774

00:11:07.060 --> 00:11:07.872 middling. Phosphorylated

NOTE Confidence: 0.71488774

00:11:07.872 --> 00:11:09.496 yeah phosphorylated. MIB is

NOTE Confidence: 0.71488774

00:11:09.500 --> 00:11:11.124 that active? Is phosphorylated

NOTE Confidence: 0.71488774

00:11:11.124 --> 00:11:12.750 MIB active? Yes yes

NOTE Confidence: 0.71488774

00:11:12.750 --> 00:11:16.285 so. So that is a key phosphorylation

NOTE Confidence: 0.71488774

00:11:16.285 --> 00:11:19.110 site that's recognized by in OK. OK,

NOTE Confidence: 0.7562662

00:11:19.110 --> 00:11:21.360 so any OK is phosphorylating

NOTE Confidence: 0.7562662

00:11:21.360 --> 00:11:23.610 in activating them? Yes OK

NOTE Confidence: 0.7562662

00:11:23.610 --> 00:11:26.370 just clarifying yes and I'll talk

NOTE Confidence: 0.7562662

00:11:26.370 --> 00:11:29.460 more about that in a few slides.

NOTE Confidence: 0.7562662

00:11:29.460 --> 00:11:31.710 Yeah and so so now.

NOTE Confidence: 0.7562662

00:11:31.710 --> 00:11:34.830 How does an OK regulate nib actually what

NOTE Confidence: 0.7562662

00:11:34.830 --> 00:11:38.195 happens is that activated and OK then

NOTE Confidence: 0.7562662

00:11:38.195 --> 00:11:40.705 phosphorylates mid to ubiquitinated nib,

NOTE Confidence: 0.7562662

00:11:40.710 --> 00:11:43.860 an result in 26 S proteasome degradation.

NOTE Confidence: 0.7562662

00:11:43.860 --> 00:11:49.180 So if we have an OK and we knock it down.

NOTE Confidence: 0.7562662

00:11:49.180 --> 00:11:51.798 RPS 19 meter put extent progenitor cells.

NOTE Confidence: 0.7562662

00:11:51.800 --> 00:11:54.784 What we actually see is stabilization of MIB.

NOTE Confidence: 0.7562662

00:11:54.790 --> 00:11:57.022 So you see that the protein

NOTE Confidence: 0.7562662

00:11:57.022 --> 00:11:59.279 levels are higher and Konan OK.

NOTE Confidence: 0.7562662

00:11:59.280 --> 00:12:01.150 It's knocked down and this

NOTE Confidence: 0.7562662

00:12:01.150 --> 00:12:03.020 again this is in RPS.

NOTE Confidence: 0.7562662

00:12:03.020 --> 00:12:05.548 19 knockdown cells so you can see that

NOTE Confidence: 0.7562662

00:12:05.548 --> 00:12:08.627 if we again look at phosphorylated nip,

NOTE Confidence: 0.7562662

00:12:08.630 --> 00:12:10.681 that compared to when we knock down

NOTE Confidence: 0.7562662

00:12:10.681 --> 00:12:13.595 in OK we see higher levels of mid

NOTE Confidence: 0.7562662

00:12:13.595 --> 00:12:15.560 phosphorylation as we would expect

NOTE Confidence: 0.7562662

00:12:15.630 --> 00:12:17.930 because MIB results in degradation

NOTE Confidence: 0.7562662

00:12:17.930 --> 00:12:20.230 and OK phosphorylation results in.

NOTE Confidence: 0.7562662

00:12:20.230 --> 00:12:23.488 Amid degradation and also here only

NOTE Confidence: 0.7562662

00:12:23.488 --> 00:12:26.250 milk is ubiquitinated without an OK.

NOTE Confidence: 0.7562662

00:12:26.250 --> 00:12:28.760 So you see higher levels,

NOTE Confidence: 0.7562662

00:12:28.760 --> 00:12:32.274 so without an OK it's not degraded.

NOTE Confidence: 0.82151514

00:12:34.370 --> 00:12:37.762 So we also looked at C71 expression in

NOTE Confidence: 0.82151514

00:12:37.762 --> 00:12:40.857 this schematic schema that you see to

NOTE Confidence: 0.82151514

00:12:40.857 --> 00:12:44.208 indicate Orthop Oasis will Mark did was to

NOTE Confidence: 0.82151514

00:12:44.208 --> 00:12:47.200 isolate the C 71 positive cells and then

NOTE Confidence: 0.82151514

00:12:47.200 --> 00:12:50.070 to perform a Western blot with antibody

NOTE Confidence: 0.82151514

00:12:50.070 --> 00:12:52.849 specific for three nine, 298 on in.

NOTE Confidence: 0.82151514

00:12:52.849 --> 00:12:56.574 OK so here we see that only in C 71

NOTE Confidence: 0.82151514

00:12:56.574 --> 00:12:59.745 positive cells do we see an OK activation

NOTE Confidence: 0.82151514

00:12:59.745 --> 00:13:04.551 phosphorylation at this site but not in C 71.

NOTE Confidence: 0.82151514

00:13:04.560 --> 00:13:07.424 Get of cells and that's whether you have

NOTE Confidence: 0.82151514

00:13:07.424 --> 00:13:10.392 herpes 19 knocked down or control. Again,

NOTE Confidence: 0.82151514

00:13:10.392 --> 00:13:13.626 if we immunoprecipitated and OK in cells,

NOTE Confidence: 0.82151514

00:13:13.630 --> 00:13:16.878 either control or RPS 19 knockdown cells,

NOTE Confidence: 0.82151514

00:13:16.880 --> 00:13:19.666 we see that the phosphorylation in CD

NOTE Confidence: 0.82151514

00:13:19.666 --> 00:13:22.611 71 cells of these three substrates

NOTE Confidence: 0.82151514

00:13:22.611 --> 00:13:24.276 is is upregulated,

NOTE Confidence: 0.82151514

00:13:24.280 --> 00:13:27.514 and that's what we would expect because

NOTE Confidence: 0.82151514

00:13:27.514 --> 00:13:30.768 we get hyper activation of an OK.

NOTE Confidence: 0.82151514

00:13:30.770 --> 00:13:34.322 So this is only again in C 171671

NOTE Confidence: 0.82151514

00:13:34.322 --> 00:13:37.709 positive cells but not in other lineages.

NOTE Confidence: 0.7884901

00:13:39.920 --> 00:13:42.195 Furthermore, we looked at the

NOTE Confidence: 0.7884901

00:13:42.195 --> 00:13:45.120 activation of MLK in mouse models,

NOTE Confidence: 0.7884901

00:13:45.120 --> 00:13:47.964 so we collaborated with new on

NOTE Confidence: 0.7884901

00:13:47.964 --> 00:13:50.800 Flickr at Lund University in Sweden,

NOTE Confidence: 0.7884901

00:13:50.800 --> 00:13:54.576 who provided to us stem cells from RPS.

NOTE Confidence: 0.7884901

00:13:54.580 --> 00:13:55.996 19 knockdown mice.

NOTE Confidence: 0.7884901

00:13:55.996 --> 00:13:57.884 It's catcher cycling inducible.

NOTE Confidence: 0.7884901

00:13:57.890 --> 00:14:01.674 Similarly, we examine the stem cells from RP.

NOTE Confidence: 0.7884901

00:14:01.680 --> 00:14:05.343 11 flox mice, which is provided to us by

NOTE Confidence: 0.7884901

00:14:05.343 --> 00:14:08.322 Manuel Serrano from Barcelona, Spain.

NOTE Confidence: 0.7884901

00:14:08.322 --> 00:14:09.798 This is also.

NOTE Confidence: 0.7884901

00:14:09.798 --> 00:14:12.750 Tamoxifen induced to lose one allele

NOTE Confidence: 0.7884901

00:14:12.842 --> 00:14:15.862 of RP 11 gene and what we see is in

NOTE Confidence: 0.7884901

00:14:15.953 --> 00:14:19.215 these mice with which have anemia that

NOTE Confidence: 0.7884901

00:14:19.215 --> 00:14:22.570 the activity of in OK is much higher

NOTE Confidence: 0.7884901

00:14:22.570 --> 00:14:25.569 than our control mice or minus docs.

NOTE Confidence: 0.7884901

00:14:25.570 --> 00:14:28.618 In both the RPS 19 and RPL 11

NOTE Confidence: 0.7884901

00:14:28.618 --> 00:14:30.510 knockdown mice mouse cells.

NOTE Confidence: 0.7884901

00:14:30.510 --> 00:14:32.778 We also examined patient samples and

NOTE Confidence: 0.7884901

00:14:32.778 --> 00:14:35.257 this is a collaboration with Hannah

NOTE Confidence: 0.7884901

00:14:35.257 --> 00:14:37.507 Gosda from Boston Children's Hospital.

NOTE Confidence: 0.7884901

00:14:37.510 --> 00:14:39.645 She provided to us three

NOTE Confidence: 0.7884901

00:14:39.645 --> 00:14:40.926 different patient samples.

NOTE Confidence: 0.7884901

00:14:40.930 --> 00:14:43.982 With these mutations in our PS 19

NOTE Confidence: 0.7884901

00:14:43.982 --> 00:14:47.525 Mark then examined the relative in OK

NOTE Confidence: 0.7884901

00:14:47.525 --> 00:14:50.210 kindness activity compared to healthy

NOTE Confidence: 0.7884901

00:14:50.210 --> 00:14:54.150 control cells and show that that the

NOTE Confidence: 0.7884901

00:14:54.150 --> 00:14:56.362 activity was significantly increased.

NOTE Confidence: 0.7884901

00:14:56.370 --> 00:14:57.114 And finally,

NOTE Confidence: 0.7884901

00:14:57.114 --> 00:14:58.974 in collaboration with here Mitsunaga,

NOTE Confidence: 0.7884901

00:14:58.980 --> 00:15:00.468 which he had Stanford,

NOTE Confidence: 0.7884901

00:15:00.468 --> 00:15:01.584 his postdoctoral fellow,

NOTE Confidence: 0.7884901

00:15:01.590 --> 00:15:02.670 to she developed,

NOTE Confidence: 0.7884901

00:15:02.670 --> 00:15:05.190 I PS cells from two different patients

NOTE Confidence: 0.7884901

00:15:05.259 --> 00:15:07.189 who are diagnosed at Stanford,

NOTE Confidence: 0.7884901

00:15:07.190 --> 00:15:09.055 who had the typical clinical

NOTE Confidence: 0.7884901

00:15:09.055 --> 00:15:10.920 and physical features of DVA.

NOTE Confidence: 0.7884901

00:15:10.920 --> 00:15:14.268 And one of them had an RPS 26 mutation.

NOTE Confidence: 0.7884901

00:15:14.270 --> 00:15:16.888 The other one had an unknown mutation,

NOTE Confidence: 0.7884901

00:15:16.890 --> 00:15:19.098 but in all three clones that

NOTE Confidence: 0.7884901

00:15:19.098 --> 00:15:21.360 we analyzed from I PS cells,

NOTE Confidence: 0.7884901

00:15:21.360 --> 00:15:24.041 we see hyperactivation of an OK using

NOTE Confidence: 0.7884901

00:15:24.041 --> 00:15:26.069 the substrates that I mentioned.

NOTE Confidence: 0.7884901

00:15:26.070 --> 00:15:27.978 And OK leban rector.

NOTE Confidence: 0.8358886

00:15:30.530 --> 00:15:32.616 So given this in all of the

NOTE Confidence: 0.8358886

00:15:32.616 --> 00:15:34.789 models that we examined for DBA,

NOTE Confidence: 0.8358886

00:15:34.790 --> 00:15:36.258 regardless of the mutation,

NOTE Confidence: 0.8358886

00:15:36.258 --> 00:15:39.570 we were able to see an OK activation.

NOTE Confidence: 0.8358886

00:15:39.570 --> 00:15:42.354 And so we wanted to test the hypothesis

NOTE Confidence: 0.8358886

00:15:42.354 --> 00:15:45.738 could in OK be a possible target for therapy.

NOTE Confidence: 0.8358886

00:15:45.740 --> 00:15:48.719 The way that we looked at this was to

NOTE Confidence: 0.8358886

00:15:48.719 --> 00:15:51.906 study the number of two thirty 5235 cells,

NOTE Confidence: 0.8358886

00:15:51.910 --> 00:15:53.358 which is a reflection

NOTE Confidence: 0.8358886

00:15:53.358 --> 00:15:54.444 of birthright expansion.

NOTE Confidence: 0.8358886

00:15:54.450 --> 00:15:57.717 We see that in our PS 19 knockdown cells,

NOTE Confidence: 0.8358886

00:15:57.720 --> 00:15:59.898 if we treat with sin LK.

NOTE Confidence: 0.8358886

00:15:59.900 --> 00:16:01.252 To knock down MLK,

NOTE Confidence: 0.8358886

00:16:01.252 --> 00:16:04.116 we see increase in the numbers of these

NOTE Confidence: 0.8358886

00:16:04.116 --> 00:16:06.797 are three projector cells or three cells,

NOTE Confidence: 0.8358886

00:16:06.800 --> 00:16:09.816 in contrast to absence of in OK or.

NOTE Confidence: 0.8358886

00:16:09.820 --> 00:16:12.480 Design OK or a control escape in

NOTE Confidence: 0.8358886

00:16:12.480 --> 00:16:15.443 OK and the point here is that

NOTE Confidence: 0.8358886

00:16:15.443 --> 00:16:17.603 in the patients with DPA,

NOTE Confidence: 0.8358886

00:16:17.610 --> 00:16:19.815 generally speaking they are treated

NOTE Confidence: 0.8358886

00:16:19.815 --> 00:16:22.020 with steroids or transfused in

NOTE Confidence: 0.8358886

00:16:22.095 --> 00:16:24.165 when the hemoglobin is below 8.

NOTE Confidence: 0.8358886

00:16:24.170 --> 00:16:27.506 So our goal is not to necessarily improve

NOTE Confidence: 0.8358886

00:16:27.506 --> 00:16:29.907 the hemoglobin up to normal levels,

NOTE Confidence: 0.8358886

00:16:29.910 --> 00:16:32.286 but rather to increase or decreases

NOTE Confidence: 0.8358886

00:16:32.286 --> 00:16:35.189 sufficiently so that they will no longer

NOTE Confidence: 0.8358886

00:16:35.189 --> 00:16:37.697 need story therapy or wrestle transfusions.

NOTE Confidence: 0.8358886

00:16:37.700 --> 00:16:39.750 So that's really our goal.

NOTE Confidence: 0.81782615

00:16:42.210 --> 00:16:44.508 You have a hand raise. Pat Gallagher

NOTE Confidence: 0.81782615

00:16:44.508 --> 00:16:46.470 is raising his hand. OK, yes?

NOTE Confidence: 0.8330304

00:16:50.340 --> 00:16:52.255 Let me think we have to figure

NOTE Confidence: 0.8330304

00:16:52.255 --> 00:16:53.923 out how to let him talk.

NOTE Confidence: 0.8330304

00:16:53.923 --> 00:16:56.369 Hold on one second so I can do it.

NOTE Confidence: 0.8330304

00:16:56.370 --> 00:16:58.008 I can do it. Go ahead.

NOTE Confidence: 0.8330304

00:16:58.010 --> 00:17:01.868 I'm good guys are the question I got OK?

NOTE Confidence: 0.8330304

00:17:01.870 --> 00:17:05.314 Do you put my hand down there?

NOTE Confidence: 0.8330304

00:17:05.320 --> 00:17:09.139 I don't know if I can do know how to do that.

NOTE Confidence: 0.8330304

00:17:09.140 --> 00:17:11.016 I had a question though on the

NOTE Confidence: 0.8330304

00:17:11.016 --> 00:17:12.286 previous slide you normalized

NOTE Confidence: 0.8330304

00:17:12.286 --> 00:17:13.906 the healthy control cells.

NOTE Confidence: 0.8330304

00:17:13.910 --> 00:17:16.129 Yes to 100% on the previous slide.

NOTE Confidence: 0.8330304

00:17:16.130 --> 00:17:18.731 Yeah, and had to go back and if you

NOTE Confidence: 0.8330304

00:17:18.731 --> 00:17:21.353 knock down you had the sin LK and you

NOTE Confidence: 0.8330304

00:17:21.353 --> 00:17:23.758 gave the absolute number of cells.

NOTE Confidence: 0.8330304

00:17:23.760 --> 00:17:25.986 Is that affected in the healthy controls?

NOTE Confidence: 0.8330304

00:17:25.990 --> 00:17:27.258 Well certainly with DPA

NOTE Confidence: 0.8322626

00:17:27.260 --> 00:17:29.168 patient samples there are lower numbers

NOTE Confidence: 0.8322626

00:17:29.168 --> 00:17:32.036 of cells, but in spite of this we see

NOTE Confidence: 0.8322626

00:17:32.036 --> 00:17:33.938 hyperactivation of energy. So I'm talking

NOTE Confidence: 0.8322626

00:17:33.940 --> 00:17:36.530 bout in the controls on the left. In

NOTE Confidence: 0.86921525

00:17:36.530 --> 00:17:39.418 the controls, if we knock if we if

NOTE Confidence: 0.86921525

00:17:39.418 --> 00:17:42.287 we look at the numbers of cells.

NOTE Confidence: 0.86921525

00:17:42.290 --> 00:17:45.332 I don't know if we actually have done that.

NOTE Confidence: 0.86921525

00:17:45.340 --> 00:17:48.364 I mean normally we would try to

NOTE Confidence: 0.86921525

00:17:48.364 --> 00:17:50.739 normalize the cell's cell number.

NOTE Confidence: 0.86921525

00:17:50.740 --> 00:17:53.484 To make it equal on both sides,

NOTE Confidence: 0.86921525

00:17:53.490 --> 00:17:56.634 but yet it's still we see hyperactivation of.

NOTE Confidence: 0.86921525

00:17:56.640 --> 00:18:00.168 I mean we see lower levels of an OK

NOTE Confidence: 0.87146217

00:18:00.170 --> 00:18:01.738 activation. Yeah thanks yeah.

NOTE Confidence: 0.7750263

00:18:03.020 --> 00:18:04.900 So in collaboration with Johan,

NOTE Confidence: 0.7750263

00:18:04.900 --> 00:18:07.150 we were able to look at.

NOTE Confidence: 0.7750263

00:18:07.150 --> 00:18:11.929 He gave us 8 compounds that he had screened.

NOTE Confidence: 0.7750263

00:18:11.930 --> 00:18:15.106 Using a 14,000 compound library in his DBA

NOTE Confidence: 0.7750263

00:18:15.106 --> 00:18:18.666 RPS 19 knockdown mice trying to go back,

NOTE Confidence: 0.7750263

00:18:18.670 --> 00:18:23.340 but I can't. And what we saw was

NOTE Confidence: 0.7750263

00:18:23.340 --> 00:18:25.230 these eight compounds and compounds.

NOTE Confidence: 0.7750263

00:18:25.230 --> 00:18:27.533 Six and eight were the most effective

NOTE Confidence: 0.7750263

00:18:27.533 --> 00:18:30.139 as far as their third expansion.

NOTE Confidence: 0.7750263

00:18:30.140 --> 00:18:33.542 And then if we looked at the Enoch activity,

NOTE Confidence: 0.7750263

00:18:33.550 --> 00:18:35.058 the typical substrates that

NOTE Confidence: 0.7750263

00:18:35.058 --> 00:18:36.943 we've examined in the past,

NOTE Confidence: 0.7750263

00:18:36.950 --> 00:18:39.704 we see that in these two compounds we were

NOTE Confidence: 0.7750263

00:18:39.704 --> 00:18:42.618 able to see significantly decrease activity.

NOTE Confidence: 0.7750263

00:18:42.620 --> 00:18:44.888 Now here we have our Pierce

NOTE Confidence: 0.7750263

00:18:44.888 --> 00:18:46.400 19 knockout cells again,

NOTE Confidence: 0.7750263

00:18:46.400 --> 00:18:49.793 and we see with this is with sin LK.

NOTE Confidence: 0.7750263

00:18:49.800 --> 00:18:52.474 So when we add this compound is

NOTE Confidence: 0.7750263

00:18:52.474 --> 00:18:55.337 Scituate which is a TGF beta inhibitor.

NOTE Confidence: 0.7750263

00:18:55.340 --> 00:18:58.836 Which has an OK as an off target.

NOTE Confidence: 0.7750263

00:18:58.840 --> 00:19:00.595 We see that there's improvement

NOTE Confidence: 0.7750263

00:19:00.595 --> 00:19:01.648 in other places.

NOTE Confidence: 0.7750263

00:19:01.650 --> 00:19:04.107 However, if we knock down in OK,

NOTE Confidence: 0.7750263

00:19:04.110 --> 00:19:05.860 we still see an improvement.

NOTE Confidence: 0.7750263

00:19:05.860 --> 00:19:08.317 So just by knocking down and OK,

NOTE Confidence: 0.7750263

00:19:08.320 --> 00:19:11.128 we see an improvement as we would expect,

NOTE Confidence: 0.7750263

00:19:11.130 --> 00:19:13.531 and then the treatment also in combination

NOTE Confidence: 0.7750263

00:19:13.531 --> 00:19:16.304 with an saw an OK gave about equal

NOTE Confidence: 0.7750263

00:19:16.304 --> 00:19:18.500 amount of improvement of earth places.

NOTE Confidence: 0.7750263

00:19:18.500 --> 00:19:20.678 So what the suggested to us

NOTE Confidence: 0.7750263

00:19:20.678 --> 00:19:23.059 was that the effect of this SD,

NOTE Confidence: 0.7750263

00:19:23.060 --> 00:19:24.815 two ages most most likely

NOTE Confidence: 0.7750263

00:19:24.815 --> 00:19:26.570 due primarily to an OK.

NOTE Confidence: 0.7750263

00:19:26.570 --> 00:19:28.970 So pushing up in OK activity.

NOTE Confidence: 0.7750263

00:19:28.970 --> 00:19:30.465 There's no effect of Miley

NOTE Confidence: 0.7750263

00:19:30.465 --> 00:19:31.960 sales as we previously seen.

NOTE Confidence: 0.75028515

00:19:34.370 --> 00:19:36.990 This presentation also within OK

NOTE Confidence: 0.75028515

00:19:36.990 --> 00:19:40.458 Nebbish and improves with pre sis of

NOTE Confidence: 0.75028515

00:19:40.458 --> 00:19:43.498 of our mouse models again which we had

NOTE Confidence: 0.75028515

00:19:43.589 --> 00:19:46.481 shown but turn 19119 increased with

NOTE Confidence: 0.75028515

00:19:46.481 --> 00:19:49.736 treatment of our this TGF beta inhibitor.

NOTE Confidence: 0.75028515

00:19:49.736 --> 00:19:53.365 Same with RPL 11 in the fold was

NOTE Confidence: 0.75028515

00:19:53.365 --> 00:19:56.901 different a little bit but two to three

NOTE Confidence: 0.75028515

00:19:56.901 --> 00:20:00.197 fold two year 424 fold stimulation.

NOTE Confidence: 0.75028515

00:20:00.200 --> 00:20:04.778 A production of rich white cells.

NOTE Confidence: 0.75028515

00:20:04.780 --> 00:20:07.090 We we also looked at human.

NOTE Confidence: 0.75028515

00:20:07.090 --> 00:20:09.400 Our cell models, including our PSAT,

NOTE Confidence: 0.75028515

00:20:09.400 --> 00:20:11.710 knocked down and 11 knock down.

NOTE Confidence: 0.75028515

00:20:11.710 --> 00:20:14.790 We see again Approvement at our DBA cells.

NOTE Confidence: 0.75028515

00:20:14.790 --> 00:20:17.100 No ST208 with SD too late,

NOTE Confidence: 0.75028515

00:20:17.100 --> 00:20:19.530 so we see an improvement anywhere

NOTE Confidence: 0.75028515

00:20:19.530 --> 00:20:22.798 from four and a half to about 7

NOTE Confidence: 0.75028515

00:20:22.798 --> 00:20:25.132 fold and then finally a patient

NOTE Confidence: 0.75028515

00:20:25.219 --> 00:20:27.865 sample we because he 235 levels.

NOTE Confidence: 0.75028515

00:20:27.870 --> 00:20:30.362 And found where would CSD 208 that

NOTE Confidence: 0.75028515

00:20:30.362 --> 00:20:32.535 we see increase in our ourselves

NOTE Confidence: 0.75028515

00:20:32.535 --> 00:20:34.929 with red cells and above 2 fold

NOTE Confidence: 0.75028515

00:20:35.006 --> 00:20:36.788 increase by quantitating.

NOTE Confidence: 0.75028515

00:20:36.790 --> 00:20:38.730 So how does this occur?

NOTE Confidence: 0.75028515

00:20:38.730 --> 00:20:41.458 Well one of the reasons why we think

NOTE Confidence: 0.75028515

00:20:41.458 --> 00:20:44.317 MLK may be important for treatment such

NOTE Confidence: 0.75028515

00:20:44.317 --> 00:20:47.654 as losing is that Lucy and may require

NOTE Confidence: 0.75028515

00:20:47.654 --> 00:20:50.370 which is amino acid as I mentioned

NOTE Confidence: 0.75028515

00:20:50.370 --> 00:20:52.788 before in clinical trials that it

NOTE Confidence: 0.75028515

00:20:52.788 --> 00:20:55.322 may require active mtor complex and

NOTE Confidence: 0.75028515

00:20:55.322 --> 00:20:57.866 pathways to be activated or induced.

NOTE Confidence: 0.75028515

00:20:57.870 --> 00:21:00.880 And Raptor me and this whole complex.

NOTE Confidence: 0.75028515

00:21:00.880 --> 00:21:04.618 We believe this is attached to lysosome.

NOTE Confidence: 0.75028515

00:21:04.620 --> 00:21:07.956 In the case of an OK phosphorylating Raptor,

NOTE Confidence: 0.75028515

00:21:07.960 --> 00:21:10.060 we hypothesize that this released

NOTE Confidence: 0.75028515

00:21:10.060 --> 00:21:12.560 this complex from the license zone,

NOTE Confidence: 0.75028515

00:21:12.560 --> 00:21:15.250 thereby enabling translation to occur.

NOTE Confidence: 0.75028515

00:21:15.250 --> 00:21:17.390 So Mark perform evening up

NOTE Confidence: 0.75028515

00:21:17.390 --> 00:21:19.102 fluorescent experiments where he

NOTE Confidence: 0.75028515

00:21:19.102 --> 00:21:21.067 labeled Raptor with green life,

NOTE Confidence: 0.75028515

00:21:21.070 --> 00:21:24.024 some in red and then looked at

NOTE Confidence: 0.75028515

00:21:24.024 --> 00:21:26.172 colocalization in the case of

NOTE Confidence: 0.75028515

00:21:26.172 --> 00:21:28.560 RPS Anki knockdown cells where he

NOTE Confidence: 0.75028515

00:21:28.560 --> 00:21:31.469 saw was that the Raptor was cold.

NOTE Confidence: 0.75028515

00:21:31.470 --> 00:21:34.470 Localising with their lices own.

NOTE Confidence: 0.75028515

00:21:34.470 --> 00:21:37.473 And as attached seem to be attached

NOTE Confidence: 0.75028515

00:21:37.473 --> 00:21:39.699 or interacting with each other,

NOTE Confidence: 0.75028515

00:21:39.700 --> 00:21:42.416 whereas with an OK knock down we

NOTE Confidence: 0.75028515

00:21:42.416 --> 00:21:45.119 don't see wrapped or any longer

NOTE Confidence: 0.75028515

00:21:45.119 --> 00:21:47.544 colocalizes with their license home,

NOTE Confidence: 0.75028515

00:21:47.550 --> 00:21:50.710 so this is 1 possible role model that

NOTE Confidence: 0.75028515

00:21:50.710 --> 00:21:53.768 we propose for which YNLK inhibitors

NOTE Confidence: 0.75028515

00:21:53.768 --> 00:21:57.032 might actually work together with lysine

NOTE Confidence: 0.75028515

00:21:57.118 --> 00:22:00.168 to enhance the erythropoietic effect.

NOTE Confidence: 0.75028515

00:22:00.170 --> 00:22:03.074 And this is another experiment where

NOTE Confidence: 0.75028515

00:22:03.074 --> 00:22:06.228 we basically took our cell model RPS

NOTE Confidence: 0.75028515

00:22:06.228 --> 00:22:09.296 19 and looked at the number of two

NOTE Confidence: 0.75028515

00:22:09.296 --> 00:22:12.112 CD 235 cells and you can see here.

NOTE Confidence: 0.75028515

00:22:12.120 --> 00:22:13.764 So control losing increasing

NOTE Confidence: 0.75028515

00:22:13.764 --> 00:22:16.214 orbital Boyces St 208, again RTF,

NOTE Confidence: 0.75028515

00:22:16.214 --> 00:22:18.626 beta inhibitor and the combination of

NOTE Confidence: 0.75028515

00:22:18.626 --> 00:22:21.586 the two seemed to increase even more.

NOTE Confidence: 0.75028515

00:22:21.590 --> 00:22:23.685 The effects through synergistic and

NOTE Confidence: 0.75028515

00:22:23.685 --> 00:22:26.540 synergism and then the we looked at.

NOTE Confidence: 0.75028515

00:22:26.540 --> 00:22:29.668 Also for EBT one which is a downstream

NOTE Confidence: 0.75028515

00:22:29.668 --> 00:22:32.089 target of optimizing implore.

NOTE Confidence: 0.75028515

00:22:32.090 --> 00:22:34.070 And showed that the phosphorylation

NOTE Confidence: 0.75028515

00:22:34.070 --> 00:22:36.863 was also enhanced when we combine the

NOTE Confidence: 0.75028515

00:22:36.863 --> 00:22:39.823 leucine with SD in our PS90 knockdown models.

NOTE Confidence: 0.79829687

00:22:41.880 --> 00:22:43.970 So in summary of this,

NOTE Confidence: 0.79829687

00:22:43.970 --> 00:22:46.546 part of the talk we showed that in

NOTE Confidence: 0.79829687

00:22:46.546 --> 00:22:49.276 OK is activated in every projectors

NOTE Confidence: 0.79829687

00:22:49.276 --> 00:22:52.771 from DBA patient samples as well as

NOTE Confidence: 0.79829687

00:22:52.771 --> 00:22:55.255 our other human and mouse models.

NOTE Confidence: 0.79829687

00:22:55.260 --> 00:22:57.244 Pharmacological genetic inhibition of

NOTE Confidence: 0.79829687

00:22:57.244 --> 00:22:59.724 NLC increases with regenerx expansion

NOTE Confidence: 0.79829687

00:22:59.724 --> 00:23:02.593 in our models and then OK appears to

NOTE Confidence: 0.79829687

00:23:02.593 --> 00:23:04.479 exert influence on putting translation

NOTE Confidence: 0.79829687

00:23:04.479 --> 00:23:06.957 in DBA through the mtor pathway.

NOTE Confidence: 0.79829687

00:23:06.960 --> 00:23:09.991 So our focus was next to begin

NOTE Confidence: 0.79829687

00:23:09.991 --> 00:23:12.210 to identify potential therapies.

NOTE Confidence: 0.79829687

00:23:12.210 --> 00:23:14.430 That would target in OK.

NOTE Confidence: 0.79829687

00:23:14.430 --> 00:23:16.830 Unfortunately, the SD 208 compound is

NOTE Confidence: 0.79829687

00:23:16.830 --> 00:23:19.300 not ready for clinical application.

NOTE Confidence: 0.79829687

00:23:19.300 --> 00:23:21.510 It doesn't have the appropriate

NOTE Confidence: 0.79829687

00:23:21.510 --> 00:23:22.836 physical chemical properties,

NOTE Confidence: 0.79829687

00:23:22.840 --> 00:23:23.692 including solubility,

NOTE Confidence: 0.79829687

00:23:23.692 --> 00:23:27.720 to be able to be converted to the clinic.

NOTE Confidence: 0.79829687

00:23:27.720 --> 00:23:30.576 And So what we show is that we

NOTE Confidence: 0.79829687

00:23:30.576 --> 00:23:33.978 worked with a number of mid chemistry

NOTE Confidence: 0.79829687

00:23:33.978 --> 00:23:36.573 consultants from our Spark program,

NOTE Confidence: 0.79829687

00:23:36.580 --> 00:23:40.101 which is a program at Stanford to

NOTE Confidence: 0.79829687

00:23:40.101 --> 00:23:43.480 convert projects and lab to the clinics.

NOTE Confidence: 0.79829687

00:23:43.480 --> 00:23:46.077 And were able to examine a number

NOTE Confidence: 0.79829687

00:23:46.077 --> 00:23:47.190 of these compounds,

NOTE Confidence: 0.79829687

00:23:47.190 --> 00:23:49.787 all of which have different primary targets.

NOTE Confidence: 0.79829687

00:23:49.790 --> 00:23:52.380 But we tested a number of them.

NOTE Confidence: 0.79829687

00:23:52.380 --> 00:23:54.060 This is just a representative

NOTE Confidence: 0.79829687

00:23:54.060 --> 00:23:55.740 experiment showing in OK in

NOTE Confidence: 0.79829687

00:23:55.807 --> 00:23:57.579 vitro kinase activity descend,

NOTE Confidence: 0.79829687

00:23:57.580 --> 00:23:59.575 everyone is familiar with which

NOTE Confidence: 0.79829687

00:23:59.575 --> 00:24:01.570 is a tyrosine kinase inhibitor

NOTE Confidence: 0.79829687

00:24:01.641 --> 00:24:03.507 used to treat CML and carafe,

NOTE Confidence: 0.79829687

00:24:03.510 --> 00:24:06.478 and if it would be rough inhibitor OTS.

NOTE Confidence: 0.79829687

00:24:06.480 --> 00:24:09.042 167 is a milk inhibitor suppen

NOTE Confidence: 0.79829687

00:24:09.042 --> 00:24:11.557 assertive is an important hitter in

NOTE Confidence: 0.79829687

00:24:11.557 --> 00:24:14.105 St 208 is our TGF beta inhibitor.

NOTE Confidence: 0.79829687

00:24:14.110 --> 00:24:16.240 So just to show an example,

NOTE Confidence: 0.79829687

00:24:16.240 --> 00:24:18.370 we use low and high concentrations.

NOTE Confidence: 0.79829687

00:24:18.370 --> 00:24:21.578 We able to show significant decrease in OK

NOTE Confidence: 0.79829687

00:24:21.578 --> 00:24:24.539 activity with this particular drug OTS 167.

NOTE Confidence: 0.79829687

00:24:24.540 --> 00:24:27.988 We examined some of these compounds for their

NOTE Confidence: 0.79829687

00:24:27.988 --> 00:24:31.320 ability to increased risk of Oasis in RPS,

NOTE Confidence: 0.79829687

00:24:31.320 --> 00:24:33.440 19 lockdown models and here's

NOTE Confidence: 0.79829687

00:24:33.440 --> 00:24:35.136 our TGF beta inhibitor.

NOTE Confidence: 0.79829687

00:24:35.140 --> 00:24:37.260 We see various levels of

NOTE Confidence: 0.79829687

00:24:37.260 --> 00:24:38.956 increase in Rip Oasis,

NOTE Confidence: 0.79829687

00:24:38.960 --> 00:24:41.498 but mostly due to RTS 167.

NOTE Confidence: 0.79829687

00:24:41.500 --> 00:24:43.615 We also looked for phosphorylation

NOTE Confidence: 0.79829687

00:24:43.615 --> 00:24:44.884 of Raptor again,

NOTE Confidence: 0.79829687

00:24:44.890 --> 00:24:47.170 which is the target of which

NOTE Confidence: 0.79829687

00:24:47.170 --> 00:24:50.001 is in the Mentor complex and we

NOTE Confidence: 0.79829687

00:24:50.001 --> 00:24:52.437 see that there was an increase

NOTE Confidence: 0.79829687

00:24:52.437 --> 00:24:55.129 in phosphorylation of Raptor.

NOTE Confidence: 0.79829687

00:24:55.130 --> 00:24:59.458 But decrease with OTS 167 within OK activity.

NOTE Confidence: 0.82792556

00:25:02.000 --> 00:25:04.382 So our lead compound we decided

NOTE Confidence: 0.82792556

00:25:04.382 --> 00:25:06.739 to focus on was OTS 167.

NOTE Confidence: 0.82792556

00:25:06.740 --> 00:25:08.720 This is a milk inhibitor.

NOTE Confidence: 0.82792556

00:25:08.720 --> 00:25:10.775 It's currently under being studied

NOTE Confidence: 0.82792556

00:25:10.775 --> 00:25:13.267 in Phase 1/2 clinical trials and

NOTE Confidence: 0.82792556

00:25:13.267 --> 00:25:15.092 particularly in advance to acute

NOTE Confidence: 0.82792556

00:25:15.092 --> 00:25:17.410 leukemia as well as lung cancer.

NOTE Confidence: 0.82792556

00:25:17.410 --> 00:25:20.161 The drug was developed by uncle with

NOTE Confidence: 0.82792556

00:25:20.161 --> 00:25:22.150 therapy scientists and their bins.

NOTE Confidence: 0.82792556

00:25:22.150 --> 00:25:25.382 Number of studies that show that drug is

NOTE Confidence: 0.82792556

00:25:25.382 --> 00:25:28.070 still effective when milk is knocked out,

NOTE Confidence: 0.82792556

00:25:28.070 --> 00:25:32.298 so there are clearly other targets involved.

NOTE Confidence: 0.82792556

00:25:32.300 --> 00:25:35.177 This compound was shown in RP slinky

NOTE Confidence: 0.82792556

00:25:35.177 --> 00:25:37.308 knockdown cells again to increase

NOTE Confidence: 0.82792556

00:25:37.308 --> 00:25:39.363 every thread expansion and when

NOTE Confidence: 0.82792556

00:25:39.363 --> 00:25:41.775 we combine our knockdown oven OK

NOTE Confidence: 0.82792556

00:25:41.775 --> 00:25:44.351 with this inhibitor we did not see

NOTE Confidence: 0.82792556

00:25:44.360 --> 00:25:46.348 significant increase in irithyll.

NOTE Confidence: 0.82792556

00:25:46.348 --> 00:25:49.771 Police is suggesting to us again that

NOTE Confidence: 0.82792556

00:25:49.771 --> 00:25:52.298 the primary target of OTS in this

NOTE Confidence: 0.82792556

00:25:52.298 --> 00:25:55.089 system to improve it or places in OK

NOTE Confidence: 0.82792556

00:25:55.089 --> 00:25:57.956 and this drug was dosed at 200 animal

NOTE Confidence: 0.82792556

00:25:57.956 --> 00:26:00.840 or every three days for one cycle.

NOTE Confidence: 0.74062574

00:26:04.120 --> 00:26:06.418 To Sir to to understand whether

NOTE Confidence: 0.74062574

00:26:06.418 --> 00:26:08.460 there there there was toxicity

NOTE Confidence: 0.74062574

00:26:08.460 --> 00:26:10.630 to normal or through blast.

NOTE Confidence: 0.74062574

00:26:10.630 --> 00:26:12.665 We looked at every expansion

NOTE Confidence: 0.74062574

00:26:12.665 --> 00:26:14.700 as well as DBA cells.

NOTE Confidence: 0.74062574

00:26:14.700 --> 00:26:17.584 Knockdown cells with artist 19 we see

NOTE Confidence: 0.74062574

00:26:17.584 --> 00:26:20.806 that the maximum effect was at 300 animal,

NOTE Confidence: 0.74062574

00:26:20.810 --> 00:26:23.898 but we begin to see an effect in

NOTE Confidence: 0.74062574

00:26:23.898 --> 00:26:26.907 North places as early as 30 nanomolar,

NOTE Confidence: 0.74062574

00:26:26.910 --> 00:26:28.538 which shows greater than

NOTE Confidence: 0.74062574

00:26:28.538 --> 00:26:29.759 tenfold therapeutic window.

NOTE Confidence: 0.74062574

00:26:29.760 --> 00:26:31.950 Since the IC50 and normal.

NOTE Confidence: 0.74062574

00:26:31.950 --> 00:26:34.435 Or healthy with Glass was

NOTE Confidence: 0.74062574

00:26:34.435 --> 00:26:36.423 about 480 animal are.

NOTE Confidence: 0.74062574

00:26:36.430 --> 00:26:38.901 With my lead cells we saw slightly

NOTE Confidence: 0.74062574

00:26:38.901 --> 00:26:40.939 more sensitivity of this compound.

NOTE Confidence: 0.74062574

00:26:40.940 --> 00:26:44.369 Again, we can see 30 an animal or an

NOTE Confidence: 0.74062574

00:26:44.369 --> 00:26:46.958 increase in it with the police is,

NOTE Confidence: 0.74062574

00:26:46.960 --> 00:26:48.832 but then the myeloid cell we

NOTE Confidence: 0.74062574

00:26:48.832 --> 00:26:50.638 began to see decreased more

NOTE Confidence: 0.74062574

00:26:50.638 --> 00:26:52.598 significantly around 300 nanomolar,

NOTE Confidence: 0.74062574

00:26:52.600 --> 00:26:54.910 so Even so we believe the

NOTE Confidence: 0.74062574

00:26:54.910 --> 00:26:57.110 therapeutic window is about 10 fold.

NOTE Confidence: 0.74062574

00:26:57.110 --> 00:27:02.195 And again, this is an in vitro assay system.

NOTE Confidence: 0.74062574

00:27:02.200 --> 00:27:03.466 So in conclusion,

NOTE Confidence: 0.74062574

00:27:03.466 --> 00:27:05.998 Altius 167 appears to improve our

NOTE Confidence: 0.74062574

00:27:05.998 --> 00:27:08.637 ethical thesis in our DBA models in

NOTE Confidence: 0.74062574

00:27:08.637 --> 00:27:10.790 vitro with very little toxicity.

NOTE Confidence: 0.74062574

00:27:10.790 --> 00:27:12.965 There have been previous reports

NOTE Confidence: 0.74062574

00:27:12.965 --> 00:27:15.654 in Nora Blastoma and breast cancer

NOTE Confidence: 0.74062574

00:27:15.654 --> 00:27:17.394 Xenografted mouse models treated

NOTE Confidence: 0.74062574

00:27:17.394 --> 00:27:20.671 with OTS 167 twice a week for three

NOTE Confidence: 0.74062574

00:27:20.671 --> 00:27:23.812 weeks over the course of a month or so,

NOTE Confidence: 0.74062574

00:27:23.812 --> 00:27:26.068 and none of those mice developed

NOTE Confidence: 0.74062574

00:27:26.068 --> 00:27:27.560 bone marrow toxicity.

NOTE Confidence: 0.74062574

00:27:27.560 --> 00:27:30.050 There are also other inhibitors to

NOTE Confidence: 0.74062574

00:27:30.050 --> 00:27:31.710 indicate that potentially they.

NOTE Confidence: 0.74062574

00:27:31.710 --> 00:27:33.186 May be effective,

NOTE Confidence: 0.74062574

00:27:33.186 --> 00:27:35.154 including John Conyers inhibitors

NOTE Confidence: 0.74062574

00:27:35.154 --> 00:27:37.534 and we're currently testing those

NOTE Confidence: 0.74062574

00:27:37.534 --> 00:27:39.614 and finally experiments to really

NOTE Confidence: 0.74062574

00:27:39.614 --> 00:27:41.906 test this is necessary in vivo

NOTE Confidence: 0.74062574

00:27:41.906 --> 00:27:44.181 in order for us to proceed to

NOTE Confidence: 0.74062574

00:27:44.190 --> 00:27:45.438 clinical trial stage.

NOTE Confidence: 0.79584086

00:27:47.720 --> 00:27:48.956 Another interesting observation

NOTE Confidence: 0.79584086

00:27:48.956 --> 00:27:51.840 that Mark made as far as Enoch

NOTE Confidence: 0.79584086

00:27:51.912 --> 00:27:54.438 expression is the fact that metformin,

NOTE Confidence: 0.79584086

00:27:54.440 --> 00:27:56.120 the commonly used medication

NOTE Confidence: 0.79584086

00:27:56.120 --> 00:27:57.800 for type 2 diabetes,

NOTE Confidence: 0.79584086

00:27:57.800 --> 00:28:00.320 inhibits an OK expression in small

NOTE Confidence: 0.79584086

00:28:00.320 --> 00:28:02.000 cell lung cancer cells,

NOTE Confidence: 0.79584086

00:28:02.000 --> 00:28:04.478 and this drug also improves the

NOTE Confidence: 0.79584086

00:28:04.478 --> 00:28:06.620 effect of hematopoiesis and delays

NOTE Confidence: 0.79584086

00:28:06.620 --> 00:28:08.300 tumors in Fanconi mice.

NOTE Confidence: 0.79584086

00:28:08.300 --> 00:28:10.988 This has been reported to be and metformin

NOTE Confidence: 0.79584086

00:28:10.988 --> 00:28:13.758 to be protective against aldehydes,

NOTE Confidence: 0.79584086

00:28:13.760 --> 00:28:16.544 which is one of the toxins

NOTE Confidence: 0.79584086

00:28:16.544 --> 00:28:18.400 thought to affect inhibit.

NOTE Confidence: 0.79584086

00:28:18.400 --> 00:28:20.619 He met up with stem cells and

NOTE Confidence: 0.79584086

00:28:20.619 --> 00:28:22.260 he's in this disease.

NOTE Confidence: 0.79584086

00:28:22.260 --> 00:28:24.336 There is currently a phase two

NOTE Confidence: 0.79584086

00:28:24.336 --> 00:28:26.274 trial with metformin in Fanconi

NOTE Confidence: 0.79584086

00:28:26.274 --> 00:28:28.709 patients that's being directed by

NOTE Confidence: 0.79584086

00:28:28.709 --> 00:28:30.657 Akiko Shimamura Boston Children's.

NOTE Confidence: 0.79584086

00:28:30.660 --> 00:28:33.495 So what is the mechanism by which in Oak

NOTE Confidence: 0.79584086

00:28:33.495 --> 00:28:35.488 expression is inhibited by metformin?

NOTE Confidence: 0.79584086

00:28:35.490 --> 00:28:37.602 Well, one of the things we

NOTE Confidence: 0.79584086

00:28:37.602 --> 00:28:39.629 looked at was first of all,

NOTE Confidence: 0.79584086

00:28:39.630 --> 00:28:41.615 just metformin improve the cell

NOTE Confidence: 0.79584086

00:28:41.615 --> 00:28:43.947 numbers of C235 cells and we

NOTE Confidence: 0.79584086

00:28:43.947 --> 00:28:45.837 show that both in RPS 19 RP.

NOTE Confidence: 0.79584086

00:28:45.840 --> 00:28:47.802 11 Knocked down models that it

NOTE Confidence: 0.79584086

00:28:47.802 --> 00:28:49.110 does increase the production

NOTE Confidence: 0.79584086

00:28:49.171 --> 00:28:50.667 of these research senators.

NOTE Confidence: 0.79584086

00:28:50.670 --> 00:28:51.360 In contrast,

NOTE Confidence: 0.79584086

00:28:51.360 --> 00:28:53.430 there's no effect of moderate cells.

NOTE Confidence: 0.79584086

00:28:53.430 --> 00:28:55.160 We don't see any phenotype,

NOTE Confidence: 0.79584086

00:28:55.160 --> 00:28:57.920 and then it would be a few economy.

NOTE Confidence: 0.79584086

00:28:57.920 --> 00:28:59.640 Or if you eat colonies,

NOTE Confidence: 0.79584086

00:28:59.640 --> 00:29:01.720 we see an increased number.

NOTE Confidence: 0.79584086

00:29:01.720 --> 00:29:06.346 With metformin. In contrast to controls.

NOTE Confidence: 0.79584086

00:29:06.350 --> 00:29:09.910 CD 235 arthritis sales.

NOTE Confidence: 0.79584086

00:29:09.910 --> 00:29:11.610 Metformin increases by five or

NOTE Confidence: 0.79584086

00:29:11.610 --> 00:29:13.985 six fold two to six fold and

NOTE Confidence: 0.79584086

00:29:13.985 --> 00:29:16.078 then be a few mirrors for it's

NOTE Confidence: 0.79584086

00:29:16.078 --> 00:29:18.089 also about two or three fold.

NOTE Confidence: 0.79584086

00:29:18.090 --> 00:29:19.725 We've seen increasing without no

NOTE Confidence: 0.79584086

00:29:19.725 --> 00:29:21.360 effect on the mileage ourselves.

NOTE Confidence: 0.744118

00:29:23.780 --> 00:29:26.315 The metformin also improves erythropoiesis

NOTE Confidence: 0.744118

00:29:26.315 --> 00:29:28.343 by inhibiting anoche activity.

NOTE Confidence: 0.744118

00:29:28.350 --> 00:29:31.194 Here we have again in OK

NOTE Confidence: 0.744118

00:29:31.194 --> 00:29:34.449 phosphorylation of in OK Mabel Rector.

NOTE Confidence: 0.744118

00:29:34.450 --> 00:29:38.250 In all cases we see that the metformin

NOTE Confidence: 0.744118

00:29:38.250 --> 00:29:42.659 as well as SD 208 TGF beta inhibitor

NOTE Confidence: 0.744118

00:29:42.659 --> 00:29:45.619 decreases the activity of a van.

NOTE Confidence: 0.744118

00:29:45.620 --> 00:29:48.668 OK in our PS90 knockdown model.

NOTE Confidence: 0.744118

00:29:48.670 --> 00:29:51.240 The RNA expression also decreases

NOTE Confidence: 0.744118

00:29:51.240 --> 00:29:53.810 which is interesting which is.

NOTE Confidence: 0.744118

00:29:53.810 --> 00:29:57.030 Primary mechanism by which we

NOTE Confidence: 0.744118

00:29:57.030 --> 00:30:00.250 believe metformin inhibits an OK.

NOTE Confidence: 0.744118

00:30:00.250 --> 00:30:02.080 Anorith Rd expansion we see

NOTE Confidence: 0.744118

00:30:02.080 --> 00:30:04.180 again with knockdown of RPS 19.

NOTE Confidence: 0.744118

00:30:04.180 --> 00:30:06.908 Any cells and knocked down again OK at

NOTE Confidence: 0.744118

00:30:06.908 --> 00:30:09.745 the same time we see that just knock down

NOTE Confidence: 0.744118

00:30:09.745 --> 00:30:12.748 of in OK and improves mini expansion.

NOTE Confidence: 0.744118

00:30:12.750 --> 00:30:16.017 There is replaces but also just say it not

NOTE Confidence: 0.744118

00:30:16.017 --> 00:30:19.167 kind of in OK here as well as metformin.

NOTE Confidence: 0.744118

00:30:19.170 --> 00:30:21.102 So metformin not done OK in the

NOTE Confidence: 0.744118

00:30:21.102 --> 00:30:22.915 form it again no significant

NOTE Confidence: 0.744118

00:30:22.915 --> 00:30:25.215 change which again suggested some

NOTE Confidence: 0.744118

00:30:25.215 --> 00:30:27.030 informants working through an OK.

NOTE Confidence: 0.69575894

00:30:30.090 --> 00:30:30.860 Sorry.

NOTE Confidence: 0.7946285

00:30:33.190 --> 00:30:36.032 This is a slide which shows our

NOTE Confidence: 0.7946285

00:30:36.032 --> 00:30:38.299 treatment of zebrafish models in

NOTE Confidence: 0.7946285

00:30:38.299 --> 00:30:40.719 collaboration with Scholin at UCLA.

NOTE Confidence: 0.7946285

00:30:40.720 --> 00:30:42.935 He created a DBA model

NOTE Confidence: 0.7946285

00:30:42.935 --> 00:30:44.707 using RPS 19 morpholinos.

NOTE Confidence: 0.7946285

00:30:44.710 --> 00:30:47.804 This is a phenotype of the fish.

NOTE Confidence: 0.7946285

00:30:47.810 --> 00:30:50.512 After approximately 5 days you can see

NOTE Confidence: 0.7946285

00:30:50.512 --> 00:30:52.669 that these embryos show significant

NOTE Confidence: 0.7946285

00:30:52.669 --> 00:30:54.974 anemia compared to controls with

NOTE Confidence: 0.7946285

00:30:54.974 --> 00:30:57.110 the treatment with metformin.

NOTE Confidence: 0.7946285

00:30:57.110 --> 00:30:59.994 We see that there is again increase

NOTE Confidence: 0.7946285

00:30:59.994 --> 00:31:02.292 in risk reduction as indicated

NOTE Confidence: 0.7946285

00:31:02.292 --> 00:31:04.737 by staining with Odeon sitting.

NOTE Confidence: 0.7946285

00:31:04.740 --> 00:31:06.908 Which binds to hemoglobin.

NOTE Confidence: 0.82489973

00:31:09.400 --> 00:31:11.260 So how does metformin

NOTE Confidence: 0.82489973

00:31:11.260 --> 00:31:13.120 regulate in OK expression?

NOTE Confidence: 0.82489973

00:31:13.120 --> 00:31:16.840 One of the ideas is through micro RNAs,

NOTE Confidence: 0.82489973

00:31:16.840 --> 00:31:20.032 and so Mark created a number of

NOTE Confidence: 0.82489973

00:31:20.032 --> 00:31:22.452 truncation mutants that would include

NOTE Confidence: 0.82489973

00:31:22.452 --> 00:31:25.338 include a variety of micro ironies

NOTE Confidence: 0.82489973

00:31:25.338 --> 00:31:29.029 for which we can then try to identify

NOTE Confidence: 0.82489973

00:31:29.029 --> 00:31:31.446 the specific mechanism by which

NOTE Confidence: 0.82489973

00:31:31.446 --> 00:31:34.726 metformin effects in OK expression.

NOTE Confidence: 0.82489973

00:31:34.730 --> 00:31:37.915 So this is showing the levels in

NOTE Confidence: 0.82489973

00:31:37.915 --> 00:31:41.514 humans that mirror 30 a mere 26

NOTE Confidence: 0.82489973

00:31:41.514 --> 00:31:43.654 increases with metformin treatment.

NOTE Confidence: 0.82489973

00:31:43.660 --> 00:31:47.062 So this is again in human primary

NOTE Confidence: 0.82489973

00:31:47.062 --> 00:31:50.604 cells versus mice that do not show

NOTE Confidence: 0.82489973

00:31:50.604 --> 00:31:53.580 this increase in May 26 expression.

NOTE Confidence: 0.82489973

00:31:53.580 --> 00:31:57.476 So the idea here is metformin could be

NOTE Confidence: 0.82489973

00:31:57.476 --> 00:32:00.704 inducing near 26 which binds to the

NOTE Confidence: 0.82489973

00:32:00.704 --> 00:32:03.544 three prime UTR event OK resulting

NOTE Confidence: 0.82489973

00:32:03.544 --> 00:32:07.078 in inhibition of by of expression.

NOTE Confidence: 0.82489973

00:32:07.080 --> 00:32:09.774 So this is a luciferase constant

NOTE Confidence: 0.82489973

00:32:09.774 --> 00:32:11.982 Reporter construct showing activity as

NOTE Confidence: 0.82489973

00:32:11.982 --> 00:32:14.404 a reflection of transcription of in OK.

NOTE Confidence: 0.82489973

00:32:14.410 --> 00:32:17.281 You can see that with the R in micro

NOTE Confidence: 0.82489973

00:32:17.281 --> 00:32:21.028 RNA 26 mimetic that there is we see

NOTE Confidence: 0.82489973

00:32:21.028 --> 00:32:22.957 decreased transcription also with

NOTE Confidence: 0.82489973

00:32:22.957 --> 00:32:25.806 181 which is in the same region

NOTE Confidence: 0.82489973

00:32:25.806 --> 00:32:28.248 the truncation mutant as mere 26

NOTE Confidence: 0.82489973

00:32:28.248 --> 00:32:30.690 we see a decrease in me.

NOTE Confidence: 0.82489973

00:32:30.690 --> 00:32:31.908 Yeah can you

NOTE Confidence: 0.77215475

00:32:31.910 --> 00:32:35.158 clarify what you mean by a mere 26

NOTE Confidence: 0.77215475

00:32:35.160 --> 00:32:37.310 mimetic? It's it's a similar.

NOTE Confidence: 0.77215475

00:32:37.310 --> 00:32:39.158 The structure, as a mere 26,

NOTE Confidence: 0.77215475

00:32:39.160 --> 00:32:41.635 so it's acting as if it were a mere

NOTE Confidence: 0.77215475

00:32:41.635 --> 00:32:44.730 26 and binding to that site, so I hope

NOTE Confidence: 0.8210358

00:32:44.730 --> 00:32:46.524 it's done with like with the

NOTE Confidence: 0.8210358

00:32:46.524 --> 00:32:48.430 retrovirus like with a hairpin or.

NOTE Confidence: 0.8210358

00:32:48.430 --> 00:32:51.470 Yes, I believe so, yeah.

NOTE Confidence: 0.8210358

00:32:51.470 --> 00:32:53.942 And the same thing with an OK if

NOTE Confidence: 0.8210358

00:32:53.942 --> 00:32:56.635 we we see that in OK expression

NOTE Confidence: 0.8210358

00:32:56.635 --> 00:32:59.530 at the protein level is decreased.

NOTE Confidence: 0.8210358

00:32:59.530 --> 00:33:02.236 But a sponge which basically is

NOTE Confidence: 0.8210358

00:33:02.236 --> 00:33:04.747 exactly what it described it in

NOTE Confidence: 0.8210358

00:33:04.747 --> 00:33:07.219 no longer allows me or 26 to bind

NOTE Confidence: 0.8210358

00:33:07.300 --> 00:33:09.520 to the three point Muti UTR.

NOTE Confidence: 0.8210358

00:33:09.520 --> 00:33:11.860 We see that there is again

NOTE Confidence: 0.8210358

00:33:11.860 --> 00:33:14.483 increase in OK at the Mr. NY.

NOTE Confidence: 0.8210358

00:33:14.483 --> 00:33:16.601 Also prefer at the Reporter assay

NOTE Confidence: 0.8210358

00:33:16.601 --> 00:33:18.729 and also the protein level.

NOTE Confidence: 0.8143553

00:33:19.730 --> 00:33:22.786 And also the Mirror 181 on the top

NOTE Confidence: 0.8143553

00:33:22.786 --> 00:33:25.460 didn't affect, but on the bottom did

NOTE Confidence: 0.8143553

00:33:25.460 --> 00:33:27.370 what's going on with that?

NOTE Confidence: 0.8143553

00:33:27.370 --> 00:33:29.280 Let me see for here.

NOTE Confidence: 0.8143553

00:33:29.280 --> 00:33:32.272 Yeah, I mean that's a good question whether

NOTE Confidence: 0.8143553

00:33:32.272 --> 00:33:35.511 the mere 181 it may not be a specific

NOTE Confidence: 0.8143553

00:33:35.511 --> 00:33:38.070 so that it doesn't sound consistent,

NOTE Confidence: 0.8143553

00:33:38.070 --> 00:33:39.985 because you would expect me

NOTE Confidence: 0.8143553

00:33:39.985 --> 00:33:42.270 or 181 to increase there too.

NOTE Confidence: 0.8143553

00:33:42.270 --> 00:33:44.878 So you know, not all the mirror when

NOTE Confidence: 0.8143553

00:33:44.878 --> 00:33:48.008 he I guess they could be inhibiting

NOTE Confidence: 0.8143553

00:33:48.008 --> 00:33:50.398 or blocking other mere sites.

NOTE Confidence: 0.8143553

00:33:50.400 --> 00:33:51.575 So, but that's something we

NOTE Confidence: 0.8143553

00:33:51.575 --> 00:33:53.290 need to look at more carefully,

NOTE Confidence: 0.8143553

00:33:53.290 --> 00:33:55.900 but thanks for noticing, yeah.

NOTE Confidence: 0.8143553

00:33:55.900 --> 00:33:57.800 An Emmy this metformin also

NOTE Confidence: 0.8143553

00:33:57.800 --> 00:33:58.940 mediates everything voices,

NOTE Confidence: 0.8143553

00:33:58.940 --> 00:34:01.523 so again we see the C235 increase

NOTE Confidence: 0.8143553

00:34:01.523 --> 00:34:04.260 with the mimetic that Foreman or both.

NOTE Confidence: 0.8712816

00:34:05.820 --> 00:34:06.924 Finding interrupted right

NOTE Confidence: 0.8712816

00:34:06.924 --> 00:34:08.028 'cause they're happening.

NOTE Confidence: 0.8712816

00:34:08.030 --> 00:34:10.620 So there's a question from the audience.

NOTE Confidence: 0.8712816

00:34:10.620 --> 00:34:12.490 Is the difference of metformin

NOTE Confidence: 0.8712816

00:34:12.490 --> 00:34:14.360 effect on human versus mouse

NOTE Confidence: 0.8712816

00:34:14.431 --> 00:34:16.650 near 26 due to changes in mere

NOTE Confidence: 0.8712816

00:34:16.650 --> 00:34:18.370 26 transcription or processing?

NOTE Confidence: 0.8712816

00:34:18.370 --> 00:34:20.578 Yeah, that's a really good question.

NOTE Confidence: 0.8712816

00:34:20.580 --> 00:34:23.156 I don't think we really know that.

NOTE Confidence: 0.8712816

00:34:23.160 --> 00:34:25.380 We haven't really focused on the

NOTE Confidence: 0.8712816

00:34:25.380 --> 00:34:28.064 mouse system, but we do know that it's

NOTE Confidence: 0.8712816

00:34:28.064 --> 00:34:30.653 different and you know that there are

NOTE Confidence: 0.8712816

00:34:30.653 --> 00:34:33.095 clearly differences at the genomic level

NOTE Confidence: 0.8712816

00:34:33.095 --> 00:34:35.707 and that the exact reason for that.

NOTE Confidence: 0.8712816

00:34:35.710 --> 00:34:38.734 We're not sure. So it's a good.

NOTE Confidence: 0.8712816

00:34:38.740 --> 00:34:42.120 It's a great question, yeah?

NOTE Confidence: 0.8712816

00:34:42.120 --> 00:34:44.922 So both in the mouses are

NOTE Confidence: 0.8712816

00:34:44.922 --> 00:34:46.790 in the mouse system.

NOTE Confidence: 0.8712816

00:34:46.790 --> 00:34:50.984 We see an increase turn 119 and also 235.

NOTE Confidence: 0.8712816

00:34:50.990 --> 00:34:54.502 We see increase in CD 235 R 3119

NOTE Confidence: 0.8712816

00:34:54.502 --> 00:34:57.691 the expression as you would expect

NOTE Confidence: 0.8712816

00:34:57.691 --> 00:35:00.451 goes down with metformin treatment

NOTE Confidence: 0.8712816

00:35:00.451 --> 00:35:03.719 here in our PS90 knockdown cells.

NOTE Confidence: 0.8712816

00:35:03.720 --> 00:35:04.899 So, to summarize,

NOTE Confidence: 0.8712816

00:35:04.899 --> 00:35:06.471 metformin improves with crisis

NOTE Confidence: 0.8712816

00:35:06.471 --> 00:35:08.160 in our models of DPA.

NOTE Confidence: 0.8712816

00:35:08.160 --> 00:35:10.152 It decreases in OK expression through

NOTE Confidence: 0.8712816

00:35:10.152 --> 00:35:12.760 mere 26 A and targeting mirrors is

NOTE Confidence: 0.8712816

00:35:12.760 --> 00:35:15.190 a possible approach to DBA therapy.

NOTE Confidence: 0.8712816

00:35:15.190 --> 00:35:16.765 There now companies that are

NOTE Confidence: 0.8712816

00:35:16.765 --> 00:35:18.896 trying to make medics or sponges

NOTE Confidence: 0.8712816

00:35:18.896 --> 00:35:20.369 for clinical application.

NOTE Confidence: 0.8712816

00:35:20.370 --> 00:35:22.590 Although it's very in early stages.

NOTE Confidence: 0.80600363

00:35:25.020 --> 00:35:28.296 OK, for the last part of the talk I'm

NOTE Confidence: 0.80600363

00:35:28.296 --> 00:35:32.068 going to focus on a new project that I

NOTE Confidence: 0.80600363

00:35:32.068 --> 00:35:34.680 appreciate Diane and Vanessa's input.

NOTE Confidence: 0.80600363

00:35:34.680 --> 00:35:37.688 This is a protein set B1 which is

NOTE Confidence: 0.80600363

00:35:37.688 --> 00:35:40.306 special 80 rich binding protein one

NOTE Confidence: 0.80600363

00:35:40.306 --> 00:35:43.920 and we initially did a many years ago.

NOTE Confidence: 0.80600363

00:35:43.920 --> 00:35:46.950 Actually did a RNA seek experiment

NOTE Confidence: 0.80600363

00:35:46.950 --> 00:35:50.496 with fetal liver human CD 34 positive

NOTE Confidence: 0.80600363

00:35:50.496 --> 00:35:53.842 cells that were transduced with RPS 19.

NOTE Confidence: 0.80600363

00:35:53.850 --> 00:35:56.130 Like if I were constructs and found 560

NOTE Confidence: 0.80600363

00:35:56.130 --> 00:35:58.469 or so genes that are differentially

NOTE Confidence: 0.80600363

00:35:58.469 --> 00:36:01.464 expressed and then we cross reference that

NOTE Confidence: 0.80600363

00:36:01.464 --> 00:36:04.124 with the list of genes identifying earlier.

NOTE Confidence: 0.80600363

00:36:04.130 --> 00:36:06.643 It'll poesis in a paper published by

NOTE Confidence: 0.80600363

00:36:06.643 --> 00:36:09.436 Mohan Orla and also Pat Gallagher and

NOTE Confidence: 0.80600363

00:36:09.436 --> 00:36:12.281 found about 1700 genes over lapping were

NOTE Confidence: 0.80600363

00:36:12.281 --> 00:36:14.500 about 42 genes that we then analyze

NOTE Confidence: 0.80600363

00:36:14.500 --> 00:36:17.654 in a variety of our cell model systems

NOTE Confidence: 0.80600363

00:36:17.654 --> 00:36:20.094 and hematopoietic stem cells both in

NOTE Confidence: 0.80600363

00:36:20.094 --> 00:36:22.164 control an RPS 19 knockdown cells

NOTE Confidence: 0.80600363

00:36:22.164 --> 00:36:24.748 and found that among those that were.

NOTE Confidence: 0.80600363

00:36:24.750 --> 00:36:25.600 Mostly regulated,

NOTE Confidence: 0.80600363

00:36:25.600 --> 00:36:28.575 the Sepy one was very interesting to

NOTE Confidence: 0.80600363

00:36:28.575 --> 00:36:31.180 us since not much had been described

NOTE Confidence: 0.80600363

00:36:31.180 --> 00:36:34.688 at all on the role of sappy one during

NOTE Confidence: 0.80600363

00:36:34.688 --> 00:36:38.100 Aritha Poesis So sappy one is a

NOTE Confidence: 0.80600363

00:36:38.100 --> 00:36:40.700 protein that basically forms chromatin

NOTE Confidence: 0.80600363

00:36:40.793 --> 00:36:43.949 loops and regulates transcription.

NOTE Confidence: 0.80600363

00:36:43.950 --> 00:36:46.155 And there's a number of their number

NOTE Confidence: 0.80600363

00:36:46.155 --> 00:36:48.729 of papers that have very nicely

NOTE Confidence: 0.80600363

00:36:48.729 --> 00:36:51.329 described its expression and rolling

NOTE Confidence: 0.80600363

00:36:51.329 --> 00:36:52.369 hematopoietic differentiation.

NOTE Confidence: 0.80600363

00:36:52.370 --> 00:36:54.375 It's moderately expressed in HS

NOTE Confidence: 0.80600363

00:36:54.375 --> 00:36:57.000 season is required for self renewal.

NOTE Confidence: 0.80600363

00:36:57.000 --> 00:36:59.105 It's induced in lymphopoiesis and

NOTE Confidence: 0.80600363

00:36:59.105 --> 00:37:01.210 required for T cell expansion.

NOTE Confidence: 0.80600363

00:37:01.210 --> 00:37:03.340 Knockout mouse have defects in

NOTE Confidence: 0.80600363

00:37:03.340 --> 00:37:05.044 lymphopoiesis and then downregulation

NOTE Confidence: 0.80600363

00:37:05.044 --> 00:37:05.840 in Milo.

NOTE Confidence: 0.80600363

00:37:05.840 --> 00:37:08.666 Police has been demonstrated in its

NOTE Confidence: 0.80600363

00:37:08.666 --> 00:37:11.722 requirement for PU .1 regulation in

NOTE Confidence: 0.80600363

00:37:11.722 --> 00:37:14.437 in common Milo progenitor cells.

NOTE Confidence: 0.80600363

00:37:14.440 --> 00:37:17.345 So Mark looked at sappi 1M RNA

NOTE Confidence: 0.80600363

00:37:17.345 --> 00:37:18.175 expression anarchist.

NOTE Confidence: 0.80600363

00:37:18.180 --> 00:37:19.428 19 knockdown cells,

NOTE Confidence: 0.80600363

00:37:19.428 --> 00:37:20.676 day OD five.

NOTE Confidence: 0.80600363

00:37:20.680 --> 00:37:22.790 He showed that the expression

NOTE Confidence: 0.80600363

00:37:22.790 --> 00:37:25.792 decrease more rapidly in our PS 19

NOTE Confidence: 0.80600363

00:37:25.792 --> 00:37:27.827 knockdown cells compared to controls

NOTE Confidence: 0.80600363

00:37:27.827 --> 00:37:30.658 and then also at the protein level.

NOTE Confidence: 0.80600363

00:37:30.660 --> 00:37:33.257 We see that there is much more

NOTE Confidence: 0.80600363

00:37:33.257 --> 00:37:35.660 decrease in the protein levels.

NOTE Confidence: 0.80600363

00:37:35.660 --> 00:37:39.548 Is that being one day 5?

NOTE Confidence: 0.80600363

00:37:39.550 --> 00:37:42.286 The colony essays and liquid culture

NOTE Confidence: 0.80600363

00:37:42.286 --> 00:37:44.592 essays that we perform showed

NOTE Confidence: 0.80600363

00:37:44.592 --> 00:37:46.980 that in colony essays in RPS.

NOTE Confidence: 0.80600363

00:37:46.980 --> 00:37:47.828 19 knockdown.

NOTE Confidence: 0.80600363

00:37:47.828 --> 00:37:50.796 HSBC's that we did not see significant

NOTE Confidence: 0.80600363

00:37:50.796 --> 00:37:53.074 increase in colony numbers per

NOTE Confidence: 0.80600363

00:37:53.074 --> 00:37:54.850 plate with three expressions.

NOTE Confidence: 0.80600363

00:37:54.850 --> 00:37:57.196 We expressed that being one about

NOTE Confidence: 0.80600363

00:37:57.196 --> 00:38:00.089 to fold in these knockdown cells,

NOTE Confidence: 0.80600363

00:38:00.090 --> 00:38:03.142 we can see any much increase in

NOTE Confidence: 0.80600363

00:38:03.142 --> 00:38:06.210 liquid culture. However, we sell a CD.

NOTE Confidence: 0.80600363

00:38:06.210 --> 00:38:09.346 235 sales that there was increase in.

NOTE Confidence: 0.80600363

00:38:09.350 --> 00:38:12.450 With happy one overexpression.

NOTE Confidence: 0.80600363

00:38:12.450 --> 00:38:14.904 But although there was no increase

NOTE Confidence: 0.80600363

00:38:14.904 --> 00:38:16.540 in the colony numbers,

NOTE Confidence: 0.80600363

00:38:16.540 --> 00:38:19.403 we could see by visualization that on

NOTE Confidence: 0.80600363

00:38:19.403 --> 00:38:22.267 the colonies were huge for much larger,

NOTE Confidence: 0.80600363

00:38:22.270 --> 00:38:24.310 suggesting that there was a

NOTE Confidence: 0.80600363

00:38:24.310 --> 00:38:25.942 proliferation of these cells,

NOTE Confidence: 0.80600363

00:38:25.950 --> 00:38:29.214 and we did see also a normal controls,

NOTE Confidence: 0.80600363

00:38:29.220 --> 00:38:32.484 but not as dramatically as in our case,

NOTE Confidence: 0.80600363

00:38:32.490 --> 00:38:33.720 19 knockdown cells.

NOTE Confidence: 0.8144065

00:38:33.720 --> 00:38:36.990 There's also a hand up from. Yeah, it's

NOTE Confidence: 0.8144065

00:38:36.990 --> 00:38:38.630 Vince. OK, I've is.

NOTE Confidence: 0.73706543

00:38:41.950 --> 00:38:44.070 Sorry, that was sorry that

NOTE Confidence: 0.73706543

00:38:44.070 --> 00:38:45.766 was accidentally did that.

NOTE Confidence: 0.73706543

00:38:45.770 --> 00:38:47.459 Oh alright, never mind.

NOTE Confidence: 0.73706543

00:38:47.460 --> 00:38:50.225 So colony assays we see that in

NOTE Confidence: 0.73706543

00:38:50.225 --> 00:38:52.719 knockdown of sappy when in normal

NOTE Confidence: 0.73706543

00:38:52.719 --> 00:38:55.589 cells or healthy progenitors we see a

NOTE Confidence: 0.73706543

00:38:55.677 --> 00:38:58.827 slight decrease in an doxycycline or 71

NOTE Confidence: 0.73706543

00:38:58.827 --> 00:39:01.464 knockdown was 235 in liquid culture.

NOTE Confidence: 0.73706543

00:39:01.464 --> 00:39:05.112 So we do see a mild decrease in

NOTE Confidence: 0.73706543

00:39:05.112 --> 00:39:08.466 erythroid colonies and or through glass.

NOTE Confidence: 0.73706543

00:39:08.470 --> 00:39:10.070 So sad he went on.

NOTE Confidence: 0.73706543

00:39:10.070 --> 00:39:11.342 Regulation does not dramatically

NOTE Confidence: 0.73706543

00:39:11.342 --> 00:39:12.932 impact anemia phenotype in DBA.

NOTE Confidence: 0.73706543

00:39:12.940 --> 00:39:15.880 So what is the function of 71?

NOTE Confidence: 0.73706543

00:39:15.880 --> 00:39:17.588 So healthy uncommitted Mylar

NOTE Confidence: 0.73706543

00:39:17.588 --> 00:39:19.296 projectors we see that.

NOTE Confidence: 0.73706543

00:39:19.300 --> 00:39:20.294 So again,

NOTE Confidence: 0.73706543

00:39:20.294 --> 00:39:22.779 here's our colony assay results

NOTE Confidence: 0.73706543

00:39:22.779 --> 00:39:25.297 showing mild decrease in be a few.

NOTE Confidence: 0.73706543

00:39:25.300 --> 00:39:26.659 Ease the culture.

NOTE Confidence: 0.73706543

00:39:26.659 --> 00:39:29.830 The same 235 cells but in megakaryocytes

NOTE Confidence: 0.73706543

00:39:29.912 --> 00:39:32.565 regenerators we see at least a 30%

NOTE Confidence: 0.73706543

00:39:32.570 --> 00:39:35.566 decrease in with that be one knockdown.

NOTE Confidence: 0.73706543

00:39:35.570 --> 00:39:38.276 So our hypothesis at this point

NOTE Confidence: 0.73706543

00:39:38.276 --> 00:39:41.495 was that although in DPA we see

NOTE Confidence: 0.73706543

00:39:41.495 --> 00:39:43.705 a block here early erythroblast

NOTE Confidence: 0.73706543

00:39:43.705 --> 00:39:46.586 stage or seek to the 235 stage.

NOTE Confidence: 0.73706543

00:39:46.590 --> 00:39:49.060 That perhaps happy one is

NOTE Confidence: 0.73706543

00:39:49.060 --> 00:39:51.036 acting upstream of this,

NOTE Confidence: 0.73706543

00:39:51.040 --> 00:39:53.134 affecting the megakaryocyte

NOTE Confidence: 0.73706543

00:39:53.134 --> 00:39:55.228 recite progenitor stage.

NOTE Confidence: 0.73706543

00:39:55.230 --> 00:39:56.856 And so here we have again

NOTE Confidence: 0.73706543

00:39:56.856 --> 00:39:58.670 our as our PS90 knockdown.

NOTE Confidence: 0.73706543

00:39:58.670 --> 00:40:00.880 Showing calling or calling numbers.

NOTE Confidence: 0.73706543

00:40:00.880 --> 00:40:03.450 Interesting Lee with CFU GM.

NOTE Confidence: 0.73706543

00:40:03.450 --> 00:40:06.528 We see an increase in colonies.

NOTE Confidence: 0.73706543

00:40:06.530 --> 00:40:10.128 C235 expansion was noted with RPS 19.

NOTE Confidence: 0.73706543

00:40:10.130 --> 00:40:13.510 Knock down an 71.

NOTE Confidence: 0.73706543

00:40:13.510 --> 00:40:16.756 We did see also 11B expansion

NOTE Confidence: 0.73706543

00:40:16.756 --> 00:40:17.838 and overexpression.

NOTE Confidence: 0.73706543

00:40:17.840 --> 00:40:21.086 We also see CD 41 increase

NOTE Confidence: 0.73706543

00:40:21.086 --> 00:40:23.250 more even more significantly.

NOTE Confidence: 0.75487924

00:40:25.470 --> 00:40:27.410 How does this happen then?

NOTE Confidence: 0.75487924

00:40:27.410 --> 00:40:30.126 What is the mechanism downstream of that?

NOTE Confidence: 0.75487924

00:40:30.130 --> 00:40:33.082 Be? One well, one of the things one of

NOTE Confidence: 0.75487924

00:40:33.082 --> 00:40:36.027 the genes that has been very important

NOTE Confidence: 0.75487924

00:40:36.027 --> 00:40:38.659 recently has been a heat shock.

NOTE Confidence: 0.75487924

00:40:38.660 --> 00:40:40.600 70th proteins that's encoded by

NOTE Confidence: 0.75487924

00:40:40.600 --> 00:40:43.322 the gene zedex, HSP, 1A1B, and 1A,

NOTE Confidence: 0.75487924

00:40:43.322 --> 00:40:45.650 and there are three different papers.

NOTE Confidence: 0.75487924

00:40:45.650 --> 00:40:47.640 Sorry, there are three different

NOTE Confidence: 0.75487924

00:40:47.640 --> 00:40:49.630 papers that have been describing

NOTE Confidence: 0.75487924

00:40:49.693 --> 00:40:51.468 by Hermione and Leah Dacosta.

NOTE Confidence: 0.75487924

00:40:51.470 --> 00:40:55.709 The role of HSP 70 in regulating gotta one.

NOTE Confidence: 0.75487924

00:40:55.710 --> 00:40:58.076 An agency 70 is thought to interact

NOTE Confidence: 0.75487924

00:40:58.076 --> 00:41:01.107 with God and one to prevent the caspase

NOTE Confidence: 0.75487924

00:41:01.107 --> 00:41:03.400 dependent cleavage of God in one,

NOTE Confidence: 0.75487924

00:41:03.400 --> 00:41:05.350 so basically is stabilizing that

NOTE Confidence: 0.75487924

00:41:05.350 --> 00:41:08.027 complex or God or one to allow

NOTE Confidence: 0.75487924

00:41:08.027 --> 00:41:09.977 it to to to activate genes,

NOTE Confidence: 0.75487924

00:41:09.980 --> 00:41:12.868 and this is just a profile of chromosomes

NOTE Confidence: 0.75487924

00:41:12.868 --> 00:41:15.787 looking at various genes so they are not

NOTE Confidence: 0.75487924

00:41:15.787 --> 00:41:18.381 affected by sappy one or influence to

NOTE Confidence: 0.75487924

00:41:18.381 --> 00:41:20.957 rescued by setting one in the orange.

NOTE Confidence: 0.75487924

00:41:20.960 --> 00:41:24.092 So here are the two genes and if we

NOTE Confidence: 0.75487924

00:41:24.092 --> 00:41:27.046 knock down step one, we see that.

NOTE Confidence: 0.75487924

00:41:27.046 --> 00:41:29.698 This HP A1A expression goes down.

NOTE Confidence: 0.75487924

00:41:29.700 --> 00:41:30.141 Similarly,

NOTE Confidence: 0.75487924

00:41:30.141 --> 00:41:33.228 if we knock down at SABI one

NOTE Confidence: 0.75487924

00:41:33.228 --> 00:41:36.127 we see HSP one being Arnie,

NOTE Confidence: 0.75487924

00:41:36.130 --> 00:41:40.618 decreasing ansim with the protein levels.

NOTE Confidence: 0.75487924

00:41:40.620 --> 00:41:43.026 If we then look examine our

NOTE Confidence: 0.75487924

00:41:43.026 --> 00:41:44.630 peacemaking knockdown cells and

NOTE Confidence: 0.75487924

00:41:44.703 --> 00:41:46.539 then overexpress sappy one,

NOTE Confidence: 0.75487924

00:41:46.540 --> 00:41:49.438 we see that there's a rescue and

NOTE Confidence: 0.75487924

00:41:49.438 --> 00:41:52.039 the expression of these two genes

NOTE Confidence: 0.75487924

00:41:52.039 --> 00:41:55.000 and also at the protein level here.

NOTE Confidence: 0.75313586

00:41:58.080 --> 00:42:02.176 To understand what sappy one might be doing,

NOTE Confidence: 0.75313586

00:42:02.180 --> 00:42:04.740 and regulating house regulating it,

NOTE Confidence: 0.75313586

00:42:04.740 --> 00:42:07.806 just P1A1A and the HPA 1B.

NOTE Confidence: 0.75313586

00:42:07.810 --> 00:42:09.472 Mark performed chromatin

NOTE Confidence: 0.75313586

00:42:09.472 --> 00:42:11.688 immunoprecipitation assays with antibodies

NOTE Confidence: 0.75313586

00:42:11.688 --> 00:42:14.458 that are specific to these H3K914,

NOTE Confidence: 0.75313586

00:42:14.460 --> 00:42:15.996 desolation HK4 trimethylation

NOTE Confidence: 0.75313586

00:42:15.996 --> 00:42:18.044 HCC 27 five metalation,

NOTE Confidence: 0.75313586

00:42:18.050 --> 00:42:22.546 and these are the sites that set the

NOTE Confidence: 0.75313586

00:42:22.546 --> 00:42:26.730 one binds and what we see is that.

NOTE Confidence: 0.75313586

00:42:26.730 --> 00:42:29.496 There's also commented peaks which that

NOTE Confidence: 0.75313586

00:42:29.496 --> 00:42:32.323 with the H3K29 acetylation and HGK

NOTE Confidence: 0.75313586

00:42:32.323 --> 00:42:35.065 4 trimethylation but not with H3K27

NOTE Confidence: 0.75313586

00:42:35.065 --> 00:42:37.382 Trimethylation. So here's our model.

NOTE Confidence: 0.75313586

00:42:37.382 --> 00:42:40.622 Here is the HSP A1A gene. Here's.

NOTE Confidence: 0.75313586

00:42:40.622 --> 00:42:43.856 Here are the seven one binding sites,

NOTE Confidence: 0.75313586

00:42:43.860 --> 00:42:47.129 and here are the predicted enhancer and

NOTE Confidence: 0.75313586

00:42:47.129 --> 00:42:49.879 promoter sites that we would expect,

NOTE Confidence: 0.75313586

00:42:49.880 --> 00:42:53.058 and so sappy one promotes the looping

NOTE Confidence: 0.75313586

00:42:53.058 --> 00:42:56.928 so that all of these will be enclosed.

NOTE Confidence: 0.75313586

00:42:56.930 --> 00:42:58.678 Doximity to facilitate transcription

NOTE Confidence: 0.75313586

00:42:58.678 --> 00:43:00.426 of these two genes,

NOTE Confidence: 0.75313586

00:43:00.430 --> 00:43:02.730 another approach that Mark Hughes

NOTE Confidence: 0.75313586

00:43:02.730 --> 00:43:05.030 was as chromatin confirmation capture

NOTE Confidence: 0.75313586

00:43:05.099 --> 00:43:07.114 essay where you basically treat

NOTE Confidence: 0.75313586

00:43:07.114 --> 00:43:08.726 the cells with formalin,

NOTE Confidence: 0.75313586

00:43:08.730 --> 00:43:11.365 then digest using restriction enzymes

NOTE Confidence: 0.75313586

00:43:11.365 --> 00:43:14.927 and then perform PCR and what he

NOTE Confidence: 0.75313586

00:43:14.927 --> 00:43:17.846 showed was that with our with our

NOTE Confidence: 0.75313586

00:43:17.846 --> 00:43:20.793 control we see two nice peaks at the

NOTE Confidence: 0.75313586

00:43:20.793 --> 00:43:22.714 sites of the Enhancement Promoter

NOTE Confidence: 0.75313586

00:43:22.714 --> 00:43:25.336 regions with RPS 19 knock down,

NOTE Confidence: 0.75313586

00:43:25.340 --> 00:43:26.258 this is blunted.

NOTE Confidence: 0.75313586

00:43:26.258 --> 00:43:28.400 With knock down as happy when we

NOTE Confidence: 0.75313586

00:43:28.471 --> 00:43:30.685 basically don't see these peaks and

NOTE Confidence: 0.75313586

00:43:30.685 --> 00:43:32.815 then with re expression of happy

NOTE Confidence: 0.75313586

00:43:32.815 --> 00:43:34.873 when we see rescue and again the

NOTE Confidence: 0.75313586

00:43:34.873 --> 00:43:37.250 peaks appear again.

NOTE Confidence: 0.75313586

00:43:37.250 --> 00:43:39.542 Another way to validate these data

NOTE Confidence: 0.75313586

00:43:39.542 --> 00:43:42.510 and look at it functionally is to

NOTE Confidence: 0.75313586

00:43:42.510 --> 00:43:46.150 perform a new technology known as Cloud Nine,

NOTE Confidence: 0.75313586

00:43:46.150 --> 00:43:48.580 and this is a approach that

NOTE Confidence: 0.75313586

00:43:48.580 --> 00:43:51.160 was developed by Kevin Wang at

NOTE Confidence: 0.75313586

00:43:51.160 --> 00:43:53.355 Stanford where you use Casper,

NOTE Confidence: 0.75313586

00:43:53.360 --> 00:43:55.904 Chris crisper CAS 9 to basically

NOTE Confidence: 0.75313586

00:43:55.904 --> 00:43:58.450 target certain sequences in the genome,

NOTE Confidence: 0.75313586

00:43:58.450 --> 00:44:00.066 treat with abscisic acid,

NOTE Confidence: 0.75313586

00:44:00.066 --> 00:44:02.086 which then forms and reversibly

NOTE Confidence: 0.75313586

00:44:02.086 --> 00:44:03.539 loop that's induced,

NOTE Confidence: 0.75313586

00:44:03.540 --> 00:44:07.516 and then you can see the effects downstream.

NOTE Confidence: 0.75313586

00:44:07.520 --> 00:44:09.896 So this is what Mark did and this

NOTE Confidence: 0.75313586

00:44:09.896 --> 00:44:12.130 is again showing our sappy one,

NOTE Confidence: 0.75313586

00:44:12.130 --> 00:44:13.442 and the various promoters

NOTE Confidence: 0.75313586

00:44:13.442 --> 00:44:14.426 and enhancer region,

NOTE Confidence: 0.75313586

00:44:14.430 --> 00:44:16.726 and again using G Arnie dimer pairs.

NOTE Confidence: 0.75313586

00:44:16.730 --> 00:44:19.242 If you knock down S isep or sappy

NOTE Confidence: 0.75313586

00:44:19.242 --> 00:44:22.016 one treat with this, I said the one,

NOTE Confidence: 0.75313586

00:44:22.016 --> 00:44:24.020 you see that there's no expression

NOTE Confidence: 0.75313586

00:44:24.091 --> 00:44:24.958 of these two.

NOTE Confidence: 0.75313586

00:44:24.960 --> 00:44:26.944 He drop protein genes.

NOTE Confidence: 0.75313586

00:44:26.944 --> 00:44:29.920 If we then induce looping using

NOTE Confidence: 0.75313586

00:44:30.020 --> 00:44:32.318 Cloud nine at at the EP1,

NOTE Confidence: 0.75313586

00:44:32.320 --> 00:44:34.918 which is here to transducer to

NOTE Confidence: 0.75313586

00:44:34.918 --> 00:44:37.020 facilitate expression of his HSP.

NOTE Confidence: 0.75313586

00:44:37.020 --> 00:44:40.050 A1A we see that there's expression

NOTE Confidence: 0.75313586

00:44:40.050 --> 00:44:43.459 but not with the other JPA 1D.

NOTE Confidence: 0.75313586

00:44:43.460 --> 00:44:46.070 If you then loop at EP2,

NOTE Confidence: 0.75313586

00:44:46.070 --> 00:44:49.353 which is here so that the enhancer

NOTE Confidence: 0.75313586

00:44:49.353 --> 00:44:53.468 is next to the P2 for HSV one a B1B,

NOTE Confidence: 0.75313586

00:44:53.470 --> 00:44:56.122 you see that there's expression of

NOTE Confidence: 0.75313586

00:44:56.122 --> 00:44:59.559 this gene and also slightly of HPA 1A.

NOTE Confidence: 0.75313586

00:44:59.560 --> 00:45:02.892 And then finally this is just a

NOTE Confidence: 0.75313586

00:45:02.892 --> 00:45:06.139 control showing there's no induction Eugene.

NOTE Confidence: 0.75313586

00:45:06.140 --> 00:45:08.822 So correlating with that is also

NOTE Confidence: 0.75313586

00:45:08.822 --> 00:45:10.163 me P expansion.

NOTE Confidence: 0.75313586

00:45:10.170 --> 00:45:12.858 We we knocked down SFB one,

NOTE Confidence: 0.75313586

00:45:12.860 --> 00:45:15.100 but then we expressed it.

NOTE Confidence: 0.75313586

00:45:15.100 --> 00:45:16.324 We see increase.

NOTE Confidence: 0.75313586

00:45:16.324 --> 00:45:19.740 We also see it by looping of P1

NOTE Confidence: 0.75313586

00:45:19.740 --> 00:45:22.055 and P2 increase in comparison

NOTE Confidence: 0.75313586

00:45:22.055 --> 00:45:25.361 to controls and we don't see any

NOTE Confidence: 0.75313586

00:45:25.361 --> 00:45:28.539 effect on HCS or the CMP population.

NOTE Confidence: 0.75313586

00:45:28.540 --> 00:45:29.440 So can

NOTE Confidence: 0.7754213

00:45:29.440 --> 00:45:32.122 I just clarify what's the experimental

NOTE Confidence: 0.7754213

00:45:32.122 --> 00:45:34.476 design here for this expansion

NOTE Confidence: 0.7754213

00:45:34.476 --> 00:45:37.440 and the CMP right? Starting with

NOTE Confidence: 0.7978872

00:45:37.440 --> 00:45:40.037 this is looking at 34 positive CD,

NOTE Confidence: 0.7978872

00:45:40.040 --> 00:45:41.890 71 low population of cells,

NOTE Confidence: 0.7978872

00:45:41.890 --> 00:45:44.116 but they've been cultured in vitro

NOTE Confidence: 0.7978872

00:45:44.120 --> 00:45:47.460 plus and minus for how yes, all of these

NOTE Confidence: 0.7978872

00:45:47.460 --> 00:45:50.428 yeah and then treated with the cloud 9,

NOTE Confidence: 0.7978872

00:45:50.430 --> 00:45:52.536 so you're then treating with the

NOTE Confidence: 0.7978872

00:45:52.536 --> 00:45:54.787 dimer peers and then with abscisic

NOTE Confidence: 0.7978872

00:45:54.787 --> 00:45:57.110 acid to induce the looping. But

NOTE Confidence: 0.7978872

00:45:57.110 --> 00:45:59.330 what you're starting with me P.

NOTE Confidence: 0.7991827

00:46:00.770 --> 00:46:03.002 We're starting at the beginning and

NOTE Confidence: 0.7991827

00:46:03.002 --> 00:46:05.268 then waiting until the stage where

NOTE Confidence: 0.7991827

00:46:05.268 --> 00:46:07.386 we would see these cells appear.

NOTE Confidence: 0.7991827

00:46:07.390 --> 00:46:12.268 OK, yeah. Yeah, and then we also look.

NOTE Confidence: 0.7991827

00:46:12.270 --> 00:46:15.573 We also examine the Seppi 1S I or any

NOTE Confidence: 0.7991827

00:46:15.573 --> 00:46:18.020 induced Rizzo Blastic Buster Apolysis

NOTE Confidence: 0.7991827

00:46:18.020 --> 00:46:21.509 showed that with the looping at P1

NOTE Confidence: 0.7991827

00:46:21.509 --> 00:46:24.421 and P2 we see increasing with robust

NOTE Confidence: 0.7991827

00:46:24.421 --> 00:46:26.958 increase in CD41A positive cells

NOTE Confidence: 0.7991827

00:46:26.958 --> 00:46:31.730 but not in Salem be my light cells.

NOTE Confidence: 0.7991827

00:46:31.730 --> 00:46:33.645 The similar findings were also

NOTE Confidence: 0.7991827

00:46:33.645 --> 00:46:36.240 observed in cells that had SHRPS 19.

NOTE Confidence: 0.7991827

00:46:36.240 --> 00:46:38.496 So in our PS90 knockdown cells

NOTE Confidence: 0.7991827

00:46:38.496 --> 00:46:40.000 the same thing happened.

NOTE Confidence: 0.7991827

00:46:40.000 --> 00:46:42.639 If we loop it P1 we see

NOTE Confidence: 0.7991827

00:46:42.639 --> 00:46:44.510 the expression of HSP 1A.

NOTE Confidence: 0.7991827

00:46:44.510 --> 00:46:46.890 If we loop at P2P CHSP A1B

NOTE Confidence: 0.7991827

00:46:46.890 --> 00:46:48.650 expression and then controls,

NOTE Confidence: 0.7991827

00:46:48.650 --> 00:46:52.082 we don't see and then this is just

NOTE Confidence: 0.7991827

00:46:52.082 --> 00:46:54.462 Western blot showing the expression

NOTE Confidence: 0.7991827

00:46:54.462 --> 00:46:57.787 a day five for both of these.

NOTE Confidence: 0.7991827

00:46:57.790 --> 00:46:58.640 Experiments.

NOTE Confidence: 0.7247406

00:47:00.680 --> 00:47:02.060 So looping appears.

NOTE Confidence: 0.7247406

00:47:02.060 --> 00:47:04.360 He also rescues Happy one,

NOTE Confidence: 0.7247406

00:47:04.360 --> 00:47:06.752 defects and healthy megakaryocytes.

NOTE Confidence: 0.7247406

00:47:06.752 --> 00:47:09.144 Here's colony essays showing

NOTE Confidence: 0.7247406

00:47:09.144 --> 00:47:11.293 our looping experiments and

NOTE Confidence: 0.7247406

00:47:11.293 --> 00:47:13.891 knockdown of 71 so by looping

NOTE Confidence: 0.7247406

00:47:13.891 --> 00:47:16.727 itself we can see increasing BFUE.

NOTE Confidence: 0.7247406

00:47:16.730 --> 00:47:18.580 Nothing with the CFU GM.

NOTE Confidence: 0.7247406

00:47:18.580 --> 00:47:21.052 Also, in liquid culture we see

NOTE Confidence: 0.7247406

00:47:21.052 --> 00:47:23.736 just with looping alone we can see

NOTE Confidence: 0.7247406

00:47:23.736 --> 00:47:25.976 improvement and C 235 and 41 a,

NOTE Confidence: 0.7247406

00:47:25.980 --> 00:47:27.460 but not in CD11B.

NOTE Confidence: 0.7247406

00:47:27.460 --> 00:47:29.788 So again, that tells us that

NOTE Confidence: 0.7247406

00:47:29.788 --> 00:47:32.170 the looping is critical for the

NOTE Confidence: 0.7247406

00:47:32.249 --> 00:47:34.429 transcription of these genes to

NOTE Confidence: 0.7247406

00:47:34.429 --> 00:47:37.670 take place and also for the effects

NOTE Confidence: 0.7247406

00:47:37.670 --> 00:47:40.105 on CD235 and C41 proliferation.

NOTE Confidence: 0.7247406

00:47:40.110 --> 00:47:43.110 Finally, to link this all back to God.

NOTE Confidence: 0.7247406

00:47:43.110 --> 00:47:45.360 Oh one. As I mentioned earlier,

NOTE Confidence: 0.7247406

00:47:45.360 --> 00:47:48.104 the God one is regulated HSP 70 being

NOTE Confidence: 0.7247406

00:47:48.104 --> 00:47:50.987 available to prevent the degradation of God.

NOTE Confidence: 0.7247406

00:47:50.990 --> 00:47:51.664 Oh one.

NOTE Confidence: 0.7247406

00:47:51.664 --> 00:47:53.686 So here we see 235 positive

NOTE Confidence: 0.7247406

00:47:53.686 --> 00:47:55.859 cells E 41 positive cells,

NOTE Confidence: 0.7247406

00:47:55.860 --> 00:47:57.051 gotta one expression.

NOTE Confidence: 0.7247406

00:47:57.051 --> 00:47:59.830 And if we then take our knockdown

NOTE Confidence: 0.7247406

00:47:59.902 --> 00:48:01.487 of essay of sappy one,

NOTE Confidence: 0.7247406

00:48:01.490 --> 00:48:04.038 we see that the looping can restore

NOTE Confidence: 0.7247406

00:48:04.038 --> 00:48:06.718 the expression of God in one because

NOTE Confidence: 0.7247406

00:48:06.718 --> 00:48:08.986 now you're getting HP 70 expression.

NOTE Confidence: 0.7247406

00:48:08.990 --> 00:48:11.240 So it's able to prevent

NOTE Confidence: 0.7247406

00:48:11.240 --> 00:48:13.040 degradation of God 01.

NOTE Confidence: 0.7247406

00:48:13.040 --> 00:48:15.110 Godwin expression is just shown

NOTE Confidence: 0.7247406

00:48:15.110 --> 00:48:17.180 here showing the increase in

NOTE Confidence: 0.7247406

00:48:17.251 --> 00:48:18.935 expression correlating with with

NOTE Confidence: 0.7247406

00:48:18.935 --> 00:48:21.896 the data I just talked about in

NOTE Confidence: 0.7247406

00:48:21.896 --> 00:48:23.721 an MVP expansion also correlated

NOTE Confidence: 0.7247406

00:48:23.721 --> 00:48:26.000 with with the God one expression.

NOTE Confidence: 0.82798517

00:48:26.000 --> 00:48:28.835 So if you did a western on

NOTE Confidence: 0.82798517

00:48:28.835 --> 00:48:31.667 the top right for the HSP 70,

NOTE Confidence: 0.82798517

00:48:31.670 --> 00:48:34.100 it would parallel the gotta one.

NOTE Confidence: 0.82798517

00:48:34.100 --> 00:48:36.485 That's what we believe. Yes, yes.

NOTE Confidence: 0.82798517

00:48:36.485 --> 00:48:40.219 And so that goes back to our model where we

NOTE Confidence: 0.82798517

00:48:40.219 --> 00:48:43.418 are in normal conditions that 71 gradually

NOTE Confidence: 0.82798517

00:48:43.418 --> 00:48:46.627 decreases as cells become more committed.

NOTE Confidence: 0.82798517

00:48:46.630 --> 00:48:50.184 But we do see HP 70 increasing, which

NOTE Confidence: 0.82798517

00:48:50.184 --> 00:48:52.848 prevents gotten one from being degraded.

NOTE Confidence: 0.82798517

00:48:52.850 --> 00:48:55.508 There's some city state level there,

NOTE Confidence: 0.82798517

00:48:55.510 --> 00:48:57.286 but obviously once committed

NOTE Confidence: 0.82798517

00:48:57.286 --> 00:48:59.506 to pull through blast stage,

NOTE Confidence: 0.82798517

00:48:59.510 --> 00:49:02.168 we see significant increase in God,

NOTE Confidence: 0.82798517

00:49:02.170 --> 00:49:05.042 one in order to transduced jeans that are

NOTE Confidence: 0.82798517

00:49:05.042 --> 00:49:07.500 required for earthway differentiation,

NOTE Confidence: 0.82798517

00:49:07.500 --> 00:49:08.514 terminal differentiation.

NOTE Confidence: 0.82798517

00:49:08.514 --> 00:49:11.556 We knocked down sappy one prematurely,

NOTE Confidence: 0.82798517

00:49:11.560 --> 00:49:13.870 or, in the case of RPS,

NOTE Confidence: 0.82798517

00:49:13.870 --> 00:49:15.001 19, insufficiency, seven.

NOTE Confidence: 0.82798517

00:49:15.001 --> 00:49:18.110 That would be 1 levels are much lower,

NOTE Confidence: 0.82798517

00:49:18.110 --> 00:49:20.798 which does not allow HSP 70 P.

NOTE Confidence: 0.82798517

00:49:20.800 --> 00:49:23.495 It just be 70 should be expressed,

NOTE Confidence: 0.82798517

00:49:23.500 --> 00:49:26.188 which then blunts the expression of God.

NOTE Confidence: 0.82798517

00:49:26.190 --> 00:49:29.422 Oh, one so that we don't get the

NOTE Confidence: 0.82798517

00:49:29.422 --> 00:49:31.577 normal increase and got a wine.

NOTE Confidence: 0.82798517

00:49:31.580 --> 00:49:34.255 And we believe this contributes

NOTE Confidence: 0.82798517

00:49:34.255 --> 00:49:36.395 to the anemia phenotype.

NOTE Confidence: 0.82798517

00:49:36.400 --> 00:49:39.165 It's just a schematic of what we

NOTE Confidence: 0.82798517

00:49:39.165 --> 00:49:41.638 believe is happening in CMP, ME P.

NOTE Confidence: 0.82798517

00:49:41.638 --> 00:49:43.358 The ratio relative ratios that

NOTE Confidence: 0.82798517

00:49:43.358 --> 00:49:45.500 occur at this stage normally,

NOTE Confidence: 0.82798517

00:49:45.500 --> 00:49:47.882 and then at the risible stage

NOTE Confidence: 0.82798517

00:49:47.882 --> 00:49:50.039 would receive an abundance of God.

NOTE Confidence: 0.82798517

00:49:50.040 --> 00:49:51.560 One protein that basically

NOTE Confidence: 0.82798517

00:49:51.560 --> 00:49:53.080 overwhelms the HSP 70,

NOTE Confidence: 0.82798517

00:49:53.080 --> 00:49:55.624 but that we have enough to be able

NOTE Confidence: 0.82798517

00:49:55.624 --> 00:49:57.998 to induce Raceway specific genes,

NOTE Confidence: 0.82798517

00:49:58.000 --> 00:50:00.520 so I'd like to end there.

NOTE Confidence: 0.82798517

00:50:00.520 --> 00:50:01.645 And then just to acknowledge

NOTE Confidence: 0.82798517

00:50:01.645 --> 00:50:03.030 people who have done the work.

NOTE Confidence: 0.82798517

00:50:03.030 --> 00:50:04.578 I think I've been knowledge Mark

NOTE Confidence: 0.82798517

00:50:04.578 --> 00:50:05.897 throughout the talk as well

NOTE Confidence: 0.82798517

00:50:05.897 --> 00:50:07.542 as the other Members I lab who

NOTE Confidence: 0.82798517

00:50:07.542 --> 00:50:08.669 contributed to this project.

NOTE Confidence: 0.82798517

00:50:08.670 --> 00:50:10.178 The collaborators at Stanford

NOTE Confidence: 0.82798517

00:50:10.178 --> 00:50:12.063 and also the other collaborators

NOTE Confidence: 0.82798517

00:50:12.063 --> 00:50:13.568 that not at Stanford in,

NOTE Confidence: 0.82798517

00:50:13.570 --> 00:50:14.614 particularly Diana Vanessa,

NOTE Confidence: 0.82798517

00:50:14.614 --> 00:50:17.050 for their help in doing the experiments

NOTE Confidence: 0.82798517

00:50:17.106 --> 00:50:19.134 with happy One and Megakaryocytic with

NOTE Confidence: 0.82798517

00:50:19.134 --> 00:50:20.893 recite progenitors and the funding

NOTE Confidence: 0.82798517

00:50:20.893 --> 00:50:22.668 sources that supported this work.

NOTE Confidence: 0.82798517

00:50:22.670 --> 00:50:24.800 So I'm happy to answer questions.