WEBVTT

NOTE duration:"01:02:21"

NOTE recognizability:0.841

NOTE language:en-us

NOTE Confidence: 0.919615251111111

00:00:00.000 --> 00:00:02.132 Hey, good afternoon, everyone.

NOTE Confidence: 0.919615251111111

00:00:02.132 --> 00:00:05.506 So it's a great pleasure to introduce

NOTE Confidence: 0.919615251111111

00:00:05.506 --> 00:00:06.918 today's Grand Round speaker,

NOTE Confidence: 0.919615251111111

00:00:06.920 --> 00:00:09.125 a speaker that many of us know

NOTE Confidence: 0.919615251111111

00:00:09.125 --> 00:00:11.278 extremely well, Doctor Diane Krause,

NOTE Confidence: 0.919615251111111

00:00:11.280 --> 00:00:13.336 the Anthony N Brady Professor of

NOTE Confidence: 0.919615251111111

00:00:13.336 --> 00:00:14.920 Laboratory Medicine, Pathology

NOTE Confidence: 0.869458246666667

00:00:14.920 --> 00:00:17.360 and Cell Biology here at Yale. Diane

NOTE Confidence: 0.654115713333333

00:00:17.360 --> 00:00:20.128 did her MD PhD training at 10 at

NOTE Confidence: 0.654115713333333

00:00:20.128 --> 00:00:21.826 Penn and she also followed this

NOTE Confidence: 0.654115713333333

00:00:21.826 --> 00:00:23.318 by clinical pathology training

NOTE Confidence: 0.928602042

00:00:23.680 --> 00:00:25.760 at Penn as well. She

NOTE Confidence: 0.8557028725

00:00:25.760 --> 00:00:28.665 moved on to postdoctoral training at Johns

NOTE Confidence: 0.8557028725

00:00:28.665 --> 00:00:31.760 Hopkins and joined the Yale faculty in 1997,

NOTE Confidence: 0.8557028725

00:00:31.760 --> 00:00:34.305 and since then she's developed an

NOTE Confidence: 0.8557028725

00:00:34.305 --> 00:00:35.666 internationally recognized research

NOTE Confidence: 0.8557028725

00:00:35.666 --> 00:00:38.000 program focusing on leukemiogenesis

NOTE Confidence: 0.8557028725

00:00:38.000 --> 00:00:40.160 and hematopoietic differentiation.

NOTE Confidence: 0.8557028725

00:00:40.160 --> 00:00:43.865 Some major research areas in Diane's

NOTE Confidence: 0.8557028725

00:00:43.865 --> 00:00:46.093 group have included functionally

NOTE Confidence: 0.8557028725

00:00:46.093 --> 00:00:48.502 characterizing gene products involved

NOTE Confidence: 0.8557028725

00:00:48.502 --> 00:00:50.878 in acute megakaryoblastic leukemias,

NOTE Confidence: 0.8557028725

00:00:50.880 --> 00:00:52.880 defining transcriptional mechanisms

NOTE Confidence: 0.8557028725

00:00:52.880 --> 00:00:55.880 that regulate megacaryocyte maturation,

NOTE Confidence: 0.8557028725

00:00:55.880 --> 00:00:57.728 and elucidating factors that regulate

NOTE Confidence: 0.8557028725

00:00:57.728 --> 00:00:59.456 how the erythroid megacaryocyte

NOTE Confidence: 0.8557028725

00:00:59.456 --> 00:01:01.664 precursor cell in the bone marrow

NOTE Confidence: 0.8557028725

00:01:01.664 --> 00:01:03.560 differentiates down the erythroid versus

NOTE Confidence: 0.795772965

00:01:03.560 --> 00:01:05.480 the platelet lineage.

NOTE Confidence: 0.795772965

00:01:05.480 --> 00:01:07.422 Diane wears many hats at Yale.

NOTE Confidence: 0.795772965

00:01:07.422 --> 00:01:08.277 As many of you know,

NOTE Confidence: 0.805733884

00:01:08.280 --> 00:01:10.576 she's director of the Wine HH Stem

NOTE Confidence: 0.805733884

00:01:10.576 --> 00:01:12.080 Cell Processing Laboratory, associate

NOTE Confidence: 0.805733884

00:01:12.080 --> 00:01:14.120 director of the Blood Bank, Associate

NOTE Confidence: 0.892690221428571

00:01:14.120 --> 00:01:16.880 Director of the Yale Stem Cell Center Co,

NOTE Confidence: 0.723387105714286

00:01:16.880 --> 00:01:19.028 Director of Yale's Immunohematology

NOTE Confidence: 0.723387105714286

00:01:19.028 --> 00:01:20.639 T32 training Grant.

NOTE Confidence: 0.723387105714286

00:01:20.640 --> 00:01:23.594 And she's also the director of an

NOTE Confidence: 0.723387105714286

00:01:23.600 --> 00:01:25.518 NIHU 54 grant that has established the

NOTE Confidence: 0.758519411428571

00:01:25.560 --> 00:01:27.204 Yale Cooperative Center of

NOTE Confidence: 0.758519411428571

00:01:27.204 --> 00:01:28.437 Excellence in Hematology,

NOTE Confidence: 0.758519411428571

00:01:28.440 --> 00:01:30.760 one of five centers nationwide

NOTE Confidence: 0.876656233076923

00:01:30.760 --> 00:01:32.725 funded to increase to provide

NOTE Confidence: 0.876656233076923

00:01:32.725 --> 00:01:34.690 resources for investigators in the

NOTE Confidence: 0.876656233076923

00:01:34.753 --> 00:01:36.910 field of hematology and to provide

NOTE Confidence: 0.876656233076923

00:01:36.910 --> 00:01:39.020 training to promote, you know,

NOTE Confidence: 0.876656233076923

00:01:39.020 --> 00:01:40.560 a field of growing investigators

NOTE Confidence: 0.488559468

00:01:40.560 --> 00:01:42.880 in non legit heme. Diana

NOTE Confidence: 0.841068021666667

00:01:42.880 --> 00:01:44.525 is a recipient of numerous awards and

NOTE Confidence: 0.841068021666667

00:01:44.525 --> 00:01:46.370 just to name a few, the Klaus Meyer

NOTE Confidence: 0.841068021666667

00:01:46.370 --> 00:01:48.600 Award from Morial Sloan Kettering,

NOTE Confidence: 0.841068021666667

00:01:48.600 --> 00:01:50.399 the Tibor Greenwald Award from the American

NOTE Confidence: 0.905523254

00:01:50.400 --> 00:01:52.064 Association of Blood Banks,

NOTE Confidence: 0.905523254

00:01:52.064 --> 00:01:53.880 and she's been also inducted into

NOTE Confidence: 0.905523254

00:01:53.880 --> 00:01:55.368 the National Blood Foundation

NOTE Confidence: 0.905523254

00:01:55.368 --> 00:01:57.639 Hall of Fame. There's one local

NOTE Confidence: 0.865799702857143

00:01:57.640 --> 00:01:59.397 award that I'd really like to mention.

NOTE Confidence: 0.865799702857143

00:01:59.400 --> 00:02:01.560 In 2018, she received the Yale

NOTE Confidence: 0.865799702857143

00:02:01.560 --> 00:02:02.852 Postdoctoral Mentoring Award,

NOTE Confidence: 0.865799702857143

00:02:02.852 --> 00:02:04.830 and I think this award really

NOTE Confidence: 0.865799702857143

00:02:04.830 --> 00:02:06.906 speaks to her complete dedication

NOTE Confidence: 0.865799702857143

00:02:06.906 --> 00:02:09.928 to advance the success of women

NOTE Confidence: 0.865799702857143

00:02:09.928 --> 00:02:11.216 and those from underrepresented

NOTE Confidence: 0.865799702857143

00:02:11.216 --> 00:02:13.426 groups in science and medicine.

NOTE Confidence: 0.865799702857143

00:02:13.426 --> 00:02:15.880 She's extremely generous with her time,

NOTE Confidence: 0.865799702857143

00:02:15.880 --> 00:02:18.560 and despite her many responsibilities,

NOTE Confidence: 0.865799702857143

00:02:18.560 --> 00:02:20.832 she always finds time to serve as a

NOTE Confidence: 0.865799702857143

00:02:20.832 --> 00:02:22.994 truly dedicated mentor to a large number

NOTE Confidence: 0.865799702857143

00:02:22.994 --> 00:02:25.118 of trainees and many junior faculty,

NOTE Confidence: 0.865799702857143

00:02:25.120 --> 00:02:26.880 including Pallavi and myself.

NOTE Confidence: 0.865799702857143

00:02:26.880 --> 00:02:28.160 So we are really delighted

NOTE Confidence: 0.865799702857143

00:02:28.160 --> 00:02:29.552 that she's taken the time today

NOTE Confidence: 0.865799702857143

00:02:29.552 --> 00:02:30.800 to accept her invitation

NOTE Confidence: 0.974645148333333

00:02:30.800 --> 00:02:33.480 and present her work to you. Welcome, Dan.

NOTE Confidence: 0.891239725

00:02:38.600 --> 00:02:41.162 Thanks so much, Karen, for that really

NOTE Confidence: 0.891239725

00:02:41.162 --> 00:02:43.079 nice introduction I should have.

NOTE Confidence: 0.891239725

00:02:43.080 --> 00:02:44.074 I do have a recording of it.

NOTE Confidence: 0.891239725

00:02:44.080 --> 00:02:46.558 I can. That's me. Name my CD.

NOTE Confidence: 0.891239725

00:02:46.560 --> 00:02:47.490 I really wanted to start

NOTE Confidence: 0.891239725

00:02:47.490 --> 00:02:48.234 with the title slide.

NOTE Confidence: 0.891239725

00:02:48.240 --> 00:02:49.600 Because of this beautiful picture.

NOTE Confidence: 0.891239725

00:02:49.600 --> 00:02:52.237 I'm going to give too much in this talk.

NOTE Confidence: 0.891239725

00:02:52.240 --> 00:02:53.992 More than one should put into a one

NOTE Confidence: 0.891239725

00:02:53.992 --> 00:02:55.569 hour talk because I'm talking to

NOTE Confidence: 0.891239725

00:02:55.569 --> 00:02:57.201 pathology and I just couldn't not

NOTE Confidence: 0.891239725

00:02:57.257 --> 00:02:58.969 present some of the stuff in our lab

NOTE Confidence: 0.891239725

00:02:58.969 --> 00:03:00.936 that is just so visually beautiful

NOTE Confidence: 0.891239725

00:03:00.936 --> 00:03:03.240 and really maybe even attract some

NOTE Confidence: 0.891239725

00:03:03.240 --> 00:03:05.260 pathology trainees and faculty to

NOTE Confidence: 0.891239725

00:03:05.260 --> 00:03:07.959 collaborate on some of the the work.

NOTE Confidence: 0.891239725

00:03:07.960 --> 00:03:09.568 But I'll tell you mostly what's

NOTE Confidence: 0.891239725

00:03:09.568 --> 00:03:10.640 going on in lab.

NOTE Confidence: 0.891239725

00:03:10.640 --> 00:03:12.560 This picture is a mega karyocyte,

NOTE Confidence: 0.891239725

00:03:12.560 --> 00:03:14.440 a primary human mega karyocyte.

NOTE Confidence: 0.891239725

00:03:14.440 --> 00:03:16.414 And what you can see is that

NOTE Confidence: 0.891239725

00:03:16.414 --> 00:03:18.000 there's a lot of detail.

NOTE Confidence: 0.891239725

00:03:18.000 --> 00:03:20.358 You can even see the Golgi,

NOTE Confidence: 0.891239725

00:03:20.360 --> 00:03:23.118 the the Golgi and the endoplasmic reticulum.

NOTE Confidence: 0.891239725

00:03:23.120 --> 00:03:25.395 And what this is, is expansion microscopy.

NOTE Confidence: 0.891239725

00:03:25.400 --> 00:03:27.157 So this was taken with the confocal,

NOTE Confidence: 0.891239725

00:03:27.160 --> 00:03:28.525 but you really have a lot of

NOTE Confidence: 0.891239725

00:03:28.525 --> 00:03:30.360 the kind of detail that you can

NOTE Confidence: 0.891239725

00:03:30.360 --> 00:03:31.556 get with electron microscopy.

NOTE Confidence: 0.891239725

00:03:31.560 --> 00:03:33.240 So it's a pretty picture,

NOTE Confidence: 0.891239725

00:03:33.240 --> 00:03:34.815 but what I'll be telling you about

NOTE Confidence: 0.891239725

00:03:34.815 --> 00:03:35.923 today is hematopoiesis For those

NOTE Confidence: 0.891239725

00:03:35.923 --> 00:03:37.274 of you who don't think about this,

NOTE Confidence: 0.891239725

00:03:37.280 --> 00:03:39.072 it in our bone marrow there's a

NOTE Confidence: 0.891239725

00:03:39.072 --> 00:03:40.347 hematopoietic stem cell Like other

NOTE Confidence: 0.891239725

00:03:40.347 --> 00:03:41.943 stem cells it self renews for the

NOTE Confidence: 0.891239725

00:03:41.943 --> 00:03:43.980 life of the Organism and it can

NOTE Confidence: 0.891239725

00:03:43.980 --> 00:03:44.859 differentiate the hematopoietic

NOTE Confidence: 0.891239725

00:03:44.859 --> 00:03:46.396 stem cell differentiates into all

NOTE Confidence: 0.891239725

00:03:46.396 --> 00:03:48.580 of the cells in our peripheral blood

NOTE Confidence: 0.891239725

00:03:48.580 --> 00:03:50.200 leukocytes as well as the red,

NOTE Confidence: 0.891239725

00:03:50.200 --> 00:03:52.720 red blood cells and platelets.

NOTE Confidence: 0.891239725

00:03:52.720 --> 00:03:55.261 And my lab really focuses on this

NOTE Confidence: 0.891239725

00:03:55.261 --> 00:03:58.235 bright orange cell which we have here as MEP.

NOTE Confidence: 0.891239725

00:03:58.240 --> 00:04:00.400 I'm going to try not to talk in

NOTE Confidence: 0.891239725

00:04:00.400 --> 00:04:01.960 too many abbreviations,

NOTE Confidence: 0.891239725

00:04:01.960 --> 00:04:04.624 but the name of the MEP is a

NOTE Confidence: 0.891239725

00:04:04.624 --> 00:04:06.320 megacarycytic erythroid precursor cell,

NOTE Confidence: 0.891239725

00:04:06.320 --> 00:04:07.760 and it's kind of a mouthful,

NOTE Confidence: 0.891239725

00:04:07.760 --> 00:04:09.960 so I'll only sometimes say the whole thing.

NOTE Confidence: 0.891239725

00:04:09.960 --> 00:04:12.221 So this is the bipotent precursor of

NOTE Confidence: 0.891239725

00:04:12.221 --> 00:04:13.957 megacarycytes that make platelets and

NOTE Confidence: 0.891239725

00:04:13.957 --> 00:04:16.087 the erythroid lineage that ends up

NOTE Confidence: 0.891239725

00:04:16.087 --> 00:04:17.719 making enucleated red blood cells.

NOTE Confidence: 0.96920333

00:04:20.080 --> 00:04:21.120 And just to remind you,

NOTE Confidence: 0.96920333

00:04:21.120 --> 00:04:22.626 we make about 2,000,000 platelets and

NOTE Confidence: 0.96920333

00:04:22.626 --> 00:04:24.518 2 million red blood cells every second.

NOTE Confidence: 0.96920333

00:04:24.520 --> 00:04:27.364 So this cell is very busy making its

NOTE Confidence: 0.96920333

00:04:27.364 --> 00:04:29.474 progenitors and trying to decide.

NOTE Confidence: 0.96920333

00:04:29.480 --> 00:04:31.316 I I don't really love using the word decide,

NOTE Confidence: 0.96920333

00:04:31.320 --> 00:04:33.609 but it really helps you ask the

NOTE Confidence: 0.96920333

00:04:33.609 --> 00:04:35.319 question which lineage to go down.

NOTE Confidence: 0.96920333

00:04:35.320 --> 00:04:38.230 So what is determining the fate

NOTE Confidence: 0.96920333

00:04:38.230 --> 00:04:41.120 specification of this bipotent progenitor?

NOTE Confidence: 0.96920333

00:04:41.120 --> 00:04:42.450 Just because I wouldn't be

NOTE Confidence: 0.96920333

00:04:42.450 --> 00:04:43.514 complete without saying this,

NOTE Confidence: 0.96920333

00:04:43.520 --> 00:04:45.494 there is evidence in the literature that

NOTE Confidence: 0.96920333

00:04:45.494 --> 00:04:47.672 megacary sites can also be derived directly

NOTE Confidence: 0.96920333

00:04:47.672 --> 00:04:49.592 from a hematopoietic stem cell population.

NOTE Confidence: 0.96920333

00:04:49.600 --> 00:04:51.796 So if that is the case,

NOTE Confidence: 0.96920333

00:04:51.800 --> 00:04:53.851 then I'm not talking about that lineage

NOTE Confidence: 0.96920333

00:04:53.851 --> 00:04:55.760 to megacarycytes, I'm talking about

NOTE Confidence: 0.96920333

00:04:55.760 --> 00:04:57.960 this bipotent lineage to megacarycytes.

NOTE Confidence: 0.96920333

00:04:57.960 --> 00:04:59.400 Why did we pick MEP?

NOTE Confidence: 0.96920333

00:04:59.400 --> 00:05:00.416 Well, first of all,

NOTE Confidence: 0.96920333

00:05:00.416 --> 00:05:02.286 it's a model of bipotent fate specification

NOTE Confidence: 0.96920333

00:05:02.286 --> 00:05:04.288 which is important in all of the

NOTE Confidence: 0.96920333

00:05:04.288 --> 00:05:06.160 stem and progenitor cell biology,

NOTE Confidence: 0.96920333

00:05:06.160 --> 00:05:09.520 tissue repair and response to injury.

NOTE Confidence: 0.96920333

00:05:09.520 --> 00:05:09.933 Secondly,

NOTE Confidence: 0.96920333

00:05:09.933 --> 00:05:11.998 it's important in regenerative medicine.

NOTE Confidence: 0.96920333

00:05:12.000 --> 00:05:13.878 As most of you are aware,

NOTE Confidence: 0.96920333

00:05:13.880 --> 00:05:15.576 the place that we get our red cells

NOTE Confidence: 0.96920333

00:05:15.576 --> 00:05:17.051 and platelets that we transfuse into

NOTE Confidence: 0.96920333

00:05:17.051 --> 00:05:18.557 patients is from healthy donors and

NOTE Confidence: 0.96920333

00:05:18.603 --> 00:05:20.115 there really aren't enough of them.

NOTE Confidence: 0.96920333

00:05:20.120 --> 00:05:22.199 And there's a huge amount of work

NOTE Confidence: 0.96920333

00:05:22.199 --> 00:05:24.277 in finding and collecting cells from

NOTE Confidence: 0.96920333

00:05:24.277 --> 00:05:26.881 healthy donors in order to maintain an

NOTE Confidence: 0.96920333

00:05:26.948 --> 00:05:29.118 adequate supply for the recipients.

NOTE Confidence: 0.96920333

00:05:29.120 --> 00:05:30.554 And sometimes we really run low

NOTE Confidence: 0.96920333

00:05:30.554 --> 00:05:32.120 on platelets in red blood cells,

NOTE Confidence: 0.96920333

00:05:32.120 --> 00:05:34.640 particularly in the last year or so.

NOTE Confidence: 0.96920333

00:05:34.640 --> 00:05:36.344 We've had several times when we're

NOTE Confidence: 0.96920333

00:05:36.344 --> 00:05:37.196 near crisis situation.

NOTE Confidence: 0.96920333

00:05:37.200 --> 00:05:38.676 So if we could figure out a way to

NOTE Confidence: 0.96920333

00:05:38.676 --> 00:05:40.600 make them in vitro, that would be great.

NOTE Confidence: 0.96920333

00:05:40.600 --> 00:05:41.160 And finally,

NOTE Confidence: 0.96920333

00:05:41.160 --> 00:05:43.200 just as potential therapeutics

NOTE Confidence: 0.96920333

00:05:43.200 --> 00:05:45.750 might be identified in erythroid

NOTE Confidence: 0.96920333

00:05:45.750 --> 00:05:48.239 and megacary acidic diseases,

NOTE Confidence: 0.96920333

00:05:48.240 --> 00:05:49.970 so how does one distinguish

NOTE Confidence: 0.96920333

00:05:49.970 --> 00:05:51.936 whether you have a bipotent MEP?

NOTE Confidence: 0.96920333

00:05:51.936 --> 00:05:54.240 What you have to do is a colony

NOTE Confidence: 0.96920333

00:05:54.304 --> 00:05:55.200 forming assay.

NOTE Confidence: 0.96920333

00:05:55.200 --> 00:05:56.677 Just if you think about a bacterium,

NOTE Confidence: 0.96920333

00:05:56.680 --> 00:05:57.760 it's going to form a colony.

NOTE Confidence: 0.96920333

00:05:57.760 --> 00:06:00.000 When we do him out of aquatic assays,

NOTE Confidence: 0.96920333

00:06:00.000 --> 00:06:01.638 we take a stemmer progenitor cell,

NOTE Confidence: 0.96920333

00:06:01.640 --> 00:06:03.328 we put it into a semi solid medium

NOTE Confidence: 0.96920333

00:06:03.328 --> 00:06:05.891 in a very dilute fashion and if that

NOTE Confidence: 0.96920333

00:06:05.891 --> 00:06:07.279 cell divides and differentiates,

NOTE Confidence: 0.96920333

00:06:07.280 --> 00:06:09.800 it's going to form a colony of cells.

NOTE Confidence: 0.96920333

00:06:09.800 --> 00:06:11.609 And what we do is we over the course

NOTE Confidence: 0.96920333

00:06:11.609 --> 00:06:13.571 of the two weeks that cell makes 2

NOTE Confidence: 0.96920333

00:06:13.571 --> 00:06:15.490 cell types with one of them being

NOTE Confidence: 0.96920333

00:06:15.490 --> 00:06:16.910 megakaryocytes and other cells of

NOTE Confidence: 0.96920333

00:06:16.910 --> 00:06:17.896 the erythroid lineage.

NOTE Confidence: 0.96920333

00:06:17.896 --> 00:06:19.984 Then the cell that started that

NOTE Confidence: 0.96920333

00:06:19.984 --> 00:06:21.199 process is the MEP,

NOTE Confidence: 0.96920333

00:06:21.200 --> 00:06:24.360 the bipotent progenitor that is

NOTE Confidence: 0.96920333

00:06:24.360 --> 00:06:25.480 the assay we used.

NOTE Confidence: 0.96920333

00:06:25.480 --> 00:06:27.952 We identified in in this paper

NOTE Confidence: 0.96920333

00:06:27.952 --> 00:06:30.751 from 2016 a really good sorting

NOTE Confidence: 0.96920333

00:06:30.751 --> 00:06:33.396 strategy for primary human MEP.

NOTE Confidence: 0.96920333

00:06:33.400 --> 00:06:35.872 What we did is we worked out the

NOTE Confidence: 0.96920333

00:06:35.872 --> 00:06:38.576 assay and then tested different flow

NOTE Confidence: 0.96920333

00:06:38.576 --> 00:06:40.510 sorting approaches to come up with

NOTE Confidence: 0.96920333

00:06:40.510 --> 00:06:43.120 the best possible way of isolating the cells.

NOTE Confidence: 0.96920333

00:06:43.120 --> 00:06:44.904 What happens is after the course of two

NOTE Confidence: 0.96920333

00:06:44.904 --> 00:06:46.678 weeks a single cell forms a colony.

NOTE Confidence: 0.96920333

00:06:46.680 --> 00:06:48.198 This is a colony of cells.

NOTE Confidence: 0.96920333

00:06:48.200 --> 00:06:49.892 It's been stained with anti glycoporin

NOTE Confidence: 0.96920333

00:06:49.892 --> 00:06:52.070 A which is a surface marker for

NOTE Confidence: 0.96920333

00:06:52.070 --> 00:06:53.394 red blood cell lineage.

NOTE Confidence: 0.96920333

00:06:53.400 --> 00:06:55.104 This and this colony is entirely

NOTE Confidence: 0.96920333

00:06:55.104 --> 00:06:56.988 made-up of cells that are committed

NOTE Confidence: 0.96920333

00:06:56.988 --> 00:06:58.360 to the erythroid lineage.

NOTE Confidence: 0.96920333

00:06:58.360 --> 00:07:00.200 Here's a colony that's stained

NOTE Confidence: 0.96920333

00:07:00.200 --> 00:07:02.040 with anti CD 41 only

NOTE Confidence: 0.748385634444445

00:07:02.040 --> 00:07:04.119 CD 41 is on the mega carry site lineage.

NOTE Confidence: 0.748385634444445

00:07:04.120 --> 00:07:06.127 So this is a colony of cells that are

NOTE Confidence: 0.748385634444445

00:07:06.127 --> 00:07:08.303 mega carry site only and then we often

NOTE Confidence: 0.748385634444445

00:07:08.303 --> 00:07:10.841 get colonies that have cells of both the

NOTE Confidence: 0.748385634444445

00:07:10.841 --> 00:07:12.511 megakaryocyte and the erythroid lineage.

NOTE Confidence: 0.748385634444445

00:07:12.520 --> 00:07:13.600 And just to be more complete,

NOTE Confidence: 0.748385634444445

00:07:13.600 --> 00:07:15.841 my lab has now switched to an assay and

NOTE Confidence: 0.748385634444445

00:07:15.841 --> 00:07:17.369 rather than using immunohistochemistry for

NOTE Confidence: 0.748385634444445

00:07:17.369 --> 00:07:20.280 glia in 41 we now do immunofluorescence.

NOTE Confidence: 0.878716792666667

00:07:22.840 --> 00:07:24.618 Based on the data obtained we now

NOTE Confidence: 0.878716792666667

00:07:24.618 --> 00:07:27.223 can get a population of primary human

NOTE Confidence: 0.878716792666667

00:07:27.223 --> 00:07:28.999 mega karyocyte erythroid progenitor

NOTE Confidence: 0.878716792666667

00:07:28.999 --> 00:07:31.191 cells where if you played 100 cells

NOTE Confidence: 0.878716792666667

00:07:31.191 --> 00:07:33.745 in a plate you get about 70 colonies

NOTE Confidence: 0.878716792666667

00:07:33.745 --> 00:07:36.355 and of those colonies about 50%

NOTE Confidence: 0.878716792666667

00:07:36.360 --> 00:07:39.066 shown here in blue are cells are

NOTE Confidence: 0.878716792666667

00:07:39.066 --> 00:07:40.952 comprised of cells with both of cells

NOTE Confidence: 0.878716792666667

00:07:40.952 --> 00:07:42.884 of both the mega karyocyte and the

NOTE Confidence: 0.878716792666667

00:07:42.884 --> 00:07:44.500 erythroid lineage with the remainder

NOTE Confidence: 0.878716792666667

00:07:44.500 --> 00:07:46.432 being erythroid only and mega only.

NOTE Confidence: 0.878716792666667

00:07:46.440 --> 00:07:48.092 We also came up with sorting strategies

NOTE Confidence: 0.878716792666667

00:07:48.092 --> 00:07:49.800 for the mega karyocyte progenitor,

NOTE Confidence: 0.878716792666667

00:07:49.800 --> 00:07:51.560 with most of the colonies are mega only

NOTE Confidence: 0.878716792666667

00:07:51.560 --> 00:07:53.573 and the erythroid progenitis under your

NOTE Confidence: 0.878716792666667

00:07:53.573 --> 00:07:55.473 similarly where they're mostly erythroid.

NOTE Confidence: 0.878716792666667

00:07:55.480 --> 00:07:56.992 One of the questions you may ask you

NOTE Confidence: 0.878716792666667

00:07:56.992 --> 00:07:58.722 may be asking yourself and will kind

NOTE Confidence: 0.878716792666667

00:07:58.722 --> 00:08:00.251 of be answered throughout the course

NOTE Confidence: 0.878716792666667

00:08:00.251 --> 00:08:01.880 of the talk is do we really have a

NOTE Confidence: 0.878716792666667

00:08:01.880 --> 00:08:03.998 good sorting strategy for the MEP?

NOTE Confidence: 0.878716792666667

00:08:04.000 --> 00:08:05.400 Because it looks like half of the

NOTE Confidence: 0.878716792666667

00:08:05.400 --> 00:08:06.757 colonies are E only and MK only.

NOTE Confidence: 0.878716792666667

00:08:06.760 --> 00:08:08.832 And what I'm going to tell you is

NOTE Confidence: 0.878716792666667

00:08:08.832 --> 00:08:10.723 that the data very strongly suggest

NOTE Confidence: 0.878716792666667

00:08:10.723 --> 00:08:13.450 that what we have is quite a pure

NOTE Confidence: 0.878716792666667

00:08:13.450 --> 00:08:15.460 population and that there is a

NOTE Confidence: 0.878716792666667

00:08:15.460 --> 00:08:17.120 probability that a bipotent cell,

NOTE Confidence: 0.878716792666667

00:08:17.120 --> 00:08:19.478 when put into the culture will,

NOTE Confidence: 0.878716792666667

00:08:19.480 --> 00:08:20.576 with the First Division,

NOTE Confidence: 0.878716792666667

00:08:20.576 --> 00:08:22.640 come up with two cells that then

NOTE Confidence: 0.878716792666667

00:08:22.640 --> 00:08:24.112 subsequently all decide Erythroid

NOTE Confidence: 0.878716792666667

00:08:24.112 --> 00:08:25.952 or subsequently all decide Meg.

NOTE Confidence: 0.878716792666667

00:08:25.960 --> 00:08:27.647 And it doesn't mean that the starting

NOTE Confidence: 0.878716792666667

00:08:27.647 --> 00:08:28.915 cell didn't have the potential

NOTE Confidence: 0.878716792666667

00:08:28.915 --> 00:08:30.160 to go down both lineages,

NOTE Confidence: 0.878716792666667

00:08:30.160 --> 00:08:33.280 and I'll try to convince you of that.

NOTE Confidence: 0.878716792666667

00:08:33.280 --> 00:08:34.972 So this enrichment of these populations

NOTE Confidence: 0.878716792666667

00:08:34.972 --> 00:08:37.073 has allowed us to study the fate

NOTE Confidence: 0.878716792666667

00:08:37.073 --> 00:08:38.301 transitions from the bipotent

NOTE Confidence: 0.878716792666667

00:08:38.301 --> 00:08:39.903 progenitor to the Meg progenitor

NOTE Confidence: 0.878716792666667

00:08:39.903 --> 00:08:41.483 and from the bipotent progenitor

NOTE Confidence: 0.878716792666667

00:08:41.483 --> 00:08:44.520 to the erythroid progenitor.

NOTE Confidence: 0.878716792666667

00:08:44.520 --> 00:08:46.812 I'm going to tell you four stories today,

NOTE Confidence: 0.878716792666667

00:08:46.812 --> 00:08:48.356 hopefully not too quickly,

NOTE Confidence: 0.878716792666667

00:08:48.360 --> 00:08:49.395 but quickly enough that I'm

NOTE Confidence: 0.878716792666667

00:08:49.395 --> 00:08:50.879 done by the end of the hour.

NOTE Confidence: 0.878716792666667

00:08:50.880 --> 00:08:53.071 The 1st is some really novel data

NOTE Confidence: 0.878716792666667

00:08:53.071 --> 00:08:56.045 that came out of our single cell RNA

NOTE Confidence: 0.878716792666667

00:08:56.045 --> 00:08:58.010 sequencing of these populations that

NOTE Confidence: 0.878716792666667

00:08:58.080 --> 00:09:00.240 revealed that the cell cycle speed

NOTE Confidence: 0.878716792666667

00:09:00.240 --> 00:09:02.916 of the MEP actually seems to predict

NOTE Confidence: 0.878716792666667

00:09:02.916 --> 00:09:05.388 whether that's going to be megacaryocyte

NOTE Confidence: 0.878716792666667

00:09:05.388 --> 00:09:07.995 output or erythroid output and that we

NOTE Confidence: 0.878716792666667

00:09:07.995 --> 00:09:10.426 can actually toggle the fate of the

NOTE Confidence: 0.878716792666667

00:09:10.426 --> 00:09:12.631 MEP by toggling its cell cycle speed.

NOTE Confidence: 0.878716792666667

00:09:12.640 --> 00:09:14.464 Then I'll tell you about the role of

NOTE Confidence: 0.878716792666667

00:09:14.464 --> 00:09:16.460 the Runks 1 transcription factor and

NOTE Confidence: 0.878716792666667

00:09:16.460 --> 00:09:18.320 how it's post translational modification

NOTE Confidence: 0.878716792666667

00:09:18.320 --> 00:09:20.479 effects MEP fate and then we'll talk about,

NOTE Confidence: 0.878716792666667

00:09:20.480 --> 00:09:22.405 we'll show show you some really cool

NOTE Confidence: 0.878716792666667

00:09:22.405 --> 00:09:24.275 data watching MEP fate specification that

NOTE Confidence: 0.878716792666667

00:09:24.275 --> 00:09:26.261 really gave us those probabilities that

NOTE Confidence: 0.878716792666667

00:09:26.261 --> 00:09:28.318 I told you about that a bipotent cell

NOTE Confidence: 0.878716792666667

00:09:28.318 --> 00:09:30.916 can form an E only or an MK only colony.

NOTE Confidence: 0.878716792666667

00:09:30.920 --> 00:09:32.160 And finally expansion microscopy

NOTE Confidence: 0.878716792666667

00:09:32.160 --> 00:09:33.400 that I already introduced

NOTE Confidence: 0.733817814444444

00:09:36.720 --> 00:09:38.997 what we did once we had fact sort facts,

NOTE Confidence: 0.733817814444444

00:09:39.000 --> 00:09:42.040 gating strategies for enriching MEP,

NOTE Confidence: 0.733817814444444

00:09:42.040 --> 00:09:44.280 Meg progenitors and erythroid progenitors.

NOTE Confidence: 0.733817814444444

00:09:44.280 --> 00:09:46.170 We also sorted the upstream common

NOTE Confidence: 0.733817814444444

00:09:46.170 --> 00:09:48.053 myeloid progenitors and we sent these

NOTE Confidence: 0.733817814444444

00:09:48.053 --> 00:09:49.757 for single cell RNA SEC analysis.

NOTE Confidence: 0.733817814444444

00:09:49.760 --> 00:09:51.112 And this was work done by Yi Shan

NOTE Confidence: 0.733817814444444

00:09:51.112 --> 00:09:53.344 Liu in the lab, an amazing post doc

NOTE Confidence: 0.733817814444444

00:09:53.344 --> 00:09:55.600 who published this work in 2018.

NOTE Confidence: 0.733817814444444

00:09:55.600 --> 00:09:57.035 What you can see when you look

NOTE Confidence: 0.733817814444444

00:09:57.035 --> 00:09:58.781 at the single cell RNA SEC and if

NOTE Confidence: 0.733817814444444

00:09:58.781 --> 00:10:00.280 you're not used to looking at this,

NOTE Confidence: 0.733817814444444

00:10:00.280 --> 00:10:03.577 the data from the individual cells has

NOTE Confidence: 0.733817814444444

00:10:03.577 --> 00:10:06.652 now been categorized into four groups.

NOTE Confidence: 0.733817814444444

00:10:06.652 --> 00:10:09.560 The CMP, the common myeloid progenitor group,

NOTE Confidence: 0.733817814444444

00:10:09.560 --> 00:10:11.600 the MEP or the Meg erythroid

NOTE Confidence: 0.733817814444444

00:10:11.600 --> 00:10:12.280 progenitor group,

NOTE Confidence: 0.733817814444444

00:10:12.280 --> 00:10:14.116 the Meg progenitors or the Meg

NOTE Confidence: 0.733817814444444

00:10:14.116 --> 00:10:15.340 committed and the erythroid

NOTE Confidence: 0.733817814444444

00:10:15.393 --> 00:10:17.237 progenitors or Erythroid committed.

NOTE Confidence: 0.733817814444444

00:10:17.240 --> 00:10:18.591 And what you can see is when

NOTE Confidence: 0.733817814444444

00:10:18.591 --> 00:10:19.880 we fact sort out these MEP,

NOTE Confidence: 0.733817814444444

00:10:19.880 --> 00:10:22.200 it's really a distinct population.

NOTE Confidence: 0.733817814444444

00:10:22.200 --> 00:10:23.680 There's a bit of a graduation to it,

NOTE Confidence: 0.733817814444444

00:10:23.680 --> 00:10:25.320 but it's a distinct population.

NOTE Confidence: 0.733817814444444

00:10:25.320 --> 00:10:28.395 It looks very different from CMPMKP or ERP,

NOTE Confidence: 0.733817814444444

00:10:28.395 --> 00:10:30.252 but it looks like it had still has

NOTE Confidence: 0.733817814444444

00:10:30.252 --> 00:10:31.827 some genes that are still on from

NOTE Confidence: 0.733817814444444

00:10:31.827 --> 00:10:33.713 the CMP that are going to be turned

NOTE Confidence: 0.733817814444444

00:10:33.713 --> 00:10:36.292 off and some genes that are on in

NOTE Confidence: 0.733817814444444

00:10:36.292 --> 00:10:38.714 erythroid and mega caries like Destin

NOTE Confidence: 0.733817814444444

00:10:38.714 --> 00:10:40.838 cells that are just coming on.

NOTE Confidence: 0.733817814444444

00:10:40.840 --> 00:10:44.319 So it really is a transitional state.

NOTE Confidence: 0.733817814444444

00:10:44.320 --> 00:10:46.770 When we looked at the gene expression

NOTE Confidence: 0.733817814444444

00:10:46.770 --> 00:10:48.912 analysis and compared MEP to the

NOTE Confidence: 0.733817814444444

00:10:48.912 --> 00:10:49.600 other populations,

NOTE Confidence: 0.733817814444444

00:10:49.600 --> 00:10:51.850 what we found that the pathways

NOTE Confidence: 0.733817814444444

00:10:51.850 --> 00:10:54.259 that were over represented in the

NOTE Confidence: 0.733817814444444

00:10:54.259 --> 00:10:55.519 differentially expressed genes

NOTE Confidence: 0.733817814444444

00:10:55.519 --> 00:10:58.080 were almost always the cell cycle.

NOTE Confidence: 0.733817814444444

00:10:58.080 --> 00:10:59.744 And so you can see it's here from

NOTE Confidence: 0.733817814444444

00:10:59.744 --> 00:11:01.555 the MEP to the Meg progenitor cell

NOTE Confidence: 0.733817814444444

00:11:01.555 --> 00:11:02.356 cycle shows up,

NOTE Confidence: 0.733817814444444

00:11:02.360 --> 00:11:04.320 from the MEP to the erythroid shows up

NOTE Confidence: 0.733817814444444

00:11:04.320 --> 00:11:06.835 and the other things were were less specific.

NOTE Confidence: 0.733817814444444

00:11:06.840 --> 00:11:08.575 We weren't entirely surprised by

NOTE Confidence: 0.733817814444444

00:11:08.575 --> 00:11:10.310 this because we had preliminary

NOTE Confidence: 0.733817814444444

00:11:10.367 --> 00:11:12.317 data that were consistent with this.

NOTE Confidence: 0.733817814444444

00:11:12.320 --> 00:11:13.958 What we had done prior to getting

NOTE Confidence: 0.733817814444444

00:11:13.958 --> 00:11:16.067 the single cell RNA C data is we had

NOTE Confidence: 0.733817814444444

00:11:16.067 --> 00:11:17.273 tried a candidate approach where

NOTE Confidence: 0.733817814444444

00:11:17.273 --> 00:11:18.797 we would add various drugs and

NOTE Confidence: 0.733817814444444

00:11:18.797 --> 00:11:21.600 cytokines to the MEP to see if it

NOTE Confidence: 0.733817814444444

00:11:21.600 --> 00:11:23.232 affected their hematopoietic output.

NOTE Confidence: 0.733817814444444

00:11:23.240 --> 00:11:25.144 We already knew that in response to

NOTE Confidence: 0.733817814444444

00:11:25.144 --> 00:11:26.799 all trans retinoic acid which goes

NOTE Confidence: 0.733817814444444

00:11:26.799 --> 00:11:28.633 to the nucleus and binds directly as

NOTE Confidence: 0.733817814444444

00:11:28.686 --> 00:11:30.396 a transcription factor on the DNA,

NOTE Confidence: 0.733817814444444

00:11:30.400 --> 00:11:33.214 that we had a dose dependent increase

NOTE Confidence: 0.733817814444444

00:11:33.214 --> 00:11:35.253 in megacaryocyte only colonies when

NOTE Confidence: 0.733817814444444

00:11:35.253 --> 00:11:36.396 we added ATRA.

NOTE Confidence: 0.733817814444444

00:11:36.400 --> 00:11:38.003 We also knew that when we added

NOTE Confidence: 0.733817814444444

00:11:38.003 --> 00:11:39.558 rapamycin which is an mtor inhibitor,

NOTE Confidence: 0.733817814444444

00:11:39.560 --> 00:11:40.622 it's affecting metabolism.

NOTE Confidence: 0.733817814444444

00:11:40.622 --> 00:11:43.601 We had a a similarly A dose dependent

NOTE Confidence: 0.733817814444444

00:11:43.601 --> 00:11:45.766 increase in megacaryocyte biased and

NOTE Confidence: 0.733817814444444

00:11:45.766 --> 00:11:48.604 what we realized is that both ATRA

NOTE Confidence: 0.733817814444444

00:11:48.604 --> 00:11:51.033 and rapamycin can slow the cell cycle.

NOTE Confidence: 0.733817814444444

00:11:51.040 --> 00:11:52.784 So we tested that.

NOTE Confidence: 0.733817814444444

00:11:52.784 --> 00:11:56.632 What we've done here is a dilution assay,

NOTE Confidence: 0.733817814444444

00:11:56.632 --> 00:11:58.480 CFSE, dilution assay,

NOTE Confidence: 0.733817814444444

00:11:58.480 --> 00:11:59.628 for those of you who are not

NOTE Confidence: 0.733817814444444

00:11:59.628 --> 00:12:00.120 familiar with this,

NOTE Confidence: 0.733817814444444

00:12:00.120 --> 00:12:01.835 you stain all your cells at time

NOTE Confidence: 0.733817814444444

00:12:01.835 --> 00:12:03.520 zero with a fluorescent dye.

NOTE Confidence: 0.733817814444444

00:12:03.520 --> 00:12:05.080 Each time the cells divide,

NOTE Confidence: 0.733817814444444

00:12:05.080 --> 00:12:06.837 they have less of the fluorescent dye.

NOTE Confidence: 0.733817814444444

00:12:06.840 --> 00:12:08.760 So the further to the left they are,

NOTE Confidence: 0.733817814444444

00:12:08.760 --> 00:12:10.720 the more division there's been.

NOTE Confidence: 0.733817814444444

00:12:10.720 --> 00:12:12.253 And what you can see is the

NOTE Confidence: 0.733817814444444

00:12:12.253 --> 00:12:13.640 controls here are shown in blue.

NOTE Confidence: 0.733817814444444

00:12:13.640 --> 00:12:14.680 When you treat with ATRA,

NOTE Confidence: 0.924257633333333

00:12:14.680 --> 00:12:16.136 there's less division. Similarly,

NOTE Confidence: 0.924257633333333

00:12:16.136 --> 00:12:17.956 when you treat with rapamycin,

NOTE Confidence: 0.924257633333333

00:12:17.960 --> 00:12:20.018 there's been less division proving that

NOTE Confidence: 0.924257633333333

00:12:20.018 --> 00:12:22.039 they're both slowing the cell cycle.

NOTE Confidence: 0.924257633333333

00:12:22.040 --> 00:12:22.905 Now I'm not necessarily talking

NOTE Confidence: 0.924257633333333

00:12:22.905 --> 00:12:24.159 about the speed of the cell cycle,

NOTE Confidence: 0.924257633333333

00:12:24.160 --> 00:12:25.918 we haven't tested that, but there's

NOTE Confidence: 0.924257633333333

00:12:25.920 --> 00:12:27.640 they're dividing less frequently.

NOTE Confidence: 0.941363015833333

00:12:29.680 --> 00:12:31.129 What we did next then is just

NOTE Confidence: 0.941363015833333

00:12:31.129 --> 00:12:32.639 add a cell cycle inhibitor.

NOTE Confidence: 0.941363015833333

00:12:32.640 --> 00:12:34.320 We used CDK 46 inhibitor.

NOTE Confidence: 0.941363015833333

00:12:34.320 --> 00:12:36.640 These cells completely stopped dividing.

NOTE Confidence: 0.941363015833333

00:12:36.640 --> 00:12:39.232 We then washed that out and put them

NOTE Confidence: 0.941363015833333

00:12:39.232 --> 00:12:42.072 into the colony assays and again saw

NOTE Confidence: 0.941363015833333

00:12:42.072 --> 00:12:44.704 this dose dependent increase in the

NOTE Confidence: 0.941363015833333

00:12:44.704 --> 00:12:46.864 mega carrier site lineage specification

NOTE Confidence: 0.941363015833333

00:12:46.864 --> 00:12:49.080 of the MEP that proved this long.

NOTE Confidence: 0.941363015833333

00:12:49.080 --> 00:12:50.960 The cell cycle gave us a Meg bias.

NOTE Confidence: 0.941363015833333

00:12:50.960 --> 00:12:52.136 But what happens if you speed

NOTE Confidence: 0.941363015833333

00:12:52.136 --> 00:12:52.920 up the cell cycle?

NOTE Confidence: 0.941363015833333

00:12:52.920 --> 00:12:54.315 Well how do you speed up the cell cycle?

NOTE Confidence: 0.941363015833333

00:12:54.320 --> 00:12:56.744 One thing is that you can

NOTE Confidence: 0.941363015833333

00:12:56.744 --> 00:12:58.288 knock down CDK inhibitors.

NOTE Confidence: 0.941363015833333

00:12:58.288 --> 00:13:00.912 The CDK is that was pretty much toxic

NOTE Confidence: 0.941363015833333

00:13:00.912 --> 00:13:03.196 to the cells and didn't turn out.

NOTE Confidence: 0.941363015833333

00:13:03.200 --> 00:13:04.761 What we ended up getting to work

NOTE Confidence: 0.941363015833333

00:13:04.761 --> 00:13:06.679 is when we over expressed cyclins.

NOTE Confidence: 0.941363015833333

00:13:06.680 --> 00:13:09.284 So we got two different vectors from

NOTE Confidence: 0.941363015833333

00:13:09.284 --> 00:13:11.160 Claudia Vaskal's group in Germany,

NOTE Confidence: 0.941363015833333

00:13:11.160 --> 00:13:13.540 one that expresses CDK 2 and cycling

NOTE Confidence: 0.941363015833333

00:13:13.540 --> 00:13:16.266 E So this is the cycling dependent

NOTE Confidence: 0.941363015833333

00:13:16.266 --> 00:13:19.006 kinase 2 and the cyclin here the cyclin

NOTE Confidence: 0.941363015833333

00:13:19.006 --> 00:13:21.053 E that activates it and separately

NOTE Confidence: 0.941363015833333

00:13:21.053 --> 00:13:23.482 the CDK four and it's cyclin CDK

NOTE Confidence: 0.941363015833333

00:13:23.482 --> 00:13:25.778 Cyclin D We call this guy 2E and

NOTE Confidence: 0.941363015833333

00:13:25.778 --> 00:13:28.320 this one 4D for obvious reasons.

NOTE Confidence: 0.941363015833333

00:13:28.320 --> 00:13:31.152 And what we found is both 2E and

NOTE Confidence: 0.941363015833333

00:13:31.152 --> 00:13:33.497 4D accelerated the cycling of MEP

NOTE Confidence: 0.941363015833333

00:13:33.497 --> 00:13:35.572 getting more more cycling in vitro.

NOTE Confidence: 0.941363015833333

00:13:35.572 --> 00:13:38.213 And when we looked at the output of

NOTE Confidence: 0.941363015833333

00:13:38.213 --> 00:13:40.741 those MEP you can see that whether we

NOTE Confidence: 0.941363015833333

00:13:40.815 --> 00:13:43.235 gave them 2E or 4D on a cell by cell

NOTE Confidence: 0.941363015833333

00:13:43.240 --> 00:13:45.480 basis now we had an erythroid bias.

NOTE Confidence: 0.941363015833333

00:13:45.480 --> 00:13:47.678 So the opposite with more cell cycle,

NOTE Confidence: 0.941363015833333

00:13:47.680 --> 00:13:49.690 more E fate specification and we

NOTE Confidence: 0.941363015833333

00:13:49.690 --> 00:13:52.586 did not see this effect if we took

NOTE Confidence: 0.941363015833333

00:13:52.586 --> 00:13:54.806 cells that were already MK committed

NOTE Confidence: 0.941363015833333

00:13:54.876 --> 00:13:57.000 or already Erythroid committed.

NOTE Confidence: 0.941363015833333

00:13:57.000 --> 00:13:58.912 So part one is when we slow the

NOTE Confidence: 0.941363015833333

00:13:58.912 --> 00:14:00.357 cell cycle we get more MKP.

NOTE Confidence: 0.941363015833333

00:14:00.360 --> 00:14:01.912 When we speed up the cell cycle we

NOTE Confidence: 0.941363015833333

00:14:01.912 --> 00:14:03.745 get more Erythroid. Why, how there?

NOTE Confidence: 0.941363015833333

00:14:03.745 --> 00:14:05.635 We have a lot of ideas.

NOTE Confidence: 0.941363015833333

00:14:05.640 --> 00:14:07.082 I'm going to show you that where

NOTE Confidence: 0.941363015833333

00:14:07.082 --> 00:14:08.782 we are in terms of answering that

NOTE Confidence: 0.941363015833333

00:14:08.782 --> 00:14:10.318 which is the runks one story

NOTE Confidence: 0.911813228695652

00:14:12.720 --> 00:14:14.763 and we I'm not showing you the data but

NOTE Confidence: 0.911813228695652

00:14:14.763 --> 00:14:16.715 we've shown that MEP actually cycle more

NOTE Confidence: 0.911813228695652

00:14:16.715 --> 00:14:18.839 slowly than both Meg or Erythroid cells.

NOTE Confidence: 0.911813228695652

00:14:18.840 --> 00:14:20.220 So that's kind of an interesting

NOTE Confidence: 0.911813228695652

00:14:20.220 --> 00:14:21.896 concept that they have to speed up

NOTE Confidence: 0.911813228695652

00:14:21.896 --> 00:14:23.318 whether they're going Meg or Erythroid,

NOTE Confidence: 0.911813228695652

00:14:23.320 --> 00:14:26.596 it's just the degree to which they speed up.

NOTE Confidence: 0.911813228695652

00:14:26.600 --> 00:14:28.472 So I want to tell you about Runx 1.

NOTE Confidence: 0.911813228695652

00:14:28.480 --> 00:14:30.776 Runx one also was revealed in our

NOTE Confidence: 0.911813228695652

00:14:30.776 --> 00:14:33.225 single cell RNA seq data and then

NOTE Confidence: 0.911813228695652

00:14:33.225 --> 00:14:35.313 subsequently in bulk RNA seq data.

NOTE Confidence: 0.911813228695652

00:14:35.320 --> 00:14:37.070 When we looked at the single cell

NOTE Confidence: 0.911813228695652

00:14:37.070 --> 00:14:39.144 RNA seq data and said what's what

NOTE Confidence: 0.911813228695652

00:14:39.144 --> 00:14:40.719 is likely regulating the genes,

NOTE Confidence: 0.911813228695652

00:14:40.720 --> 00:14:43.191 the change from MEP to MKP and

NOTE Confidence: 0.911813228695652

00:14:43.191 --> 00:14:44.680 from MEP to ERP,

NOTE Confidence: 0.911813228695652

00:14:44.680 --> 00:14:46.955 from Meg to erythroid and Meg to,

NOTE Confidence: 0.911813228695652

00:14:46.960 --> 00:14:48.976 I'm sorry, from the bipotent to the Meg

NOTE Confidence: 0.911813228695652

00:14:48.976 --> 00:14:51.120 and from the bipotent to the erythroid.

NOTE Confidence: 0.911813228695652

00:14:51.120 --> 00:14:53.424 Ronx One was the predicted transcription

NOTE Confidence: 0.911813228695652

00:14:53.424 --> 00:14:56.039 factor that would be regulating this.

NOTE Confidence: 0.911813228695652

00:14:56.040 --> 00:14:57.916 Of the genes that are down regulated

NOTE Confidence: 0.911813228695652

00:14:57.916 --> 00:15:00.231 from CMP to MEP and down regulated from

NOTE Confidence: 0.911813228695652

00:15:00.231 --> 00:15:02.360 the MEP to the erythroid progenitor,

NOTE Confidence: 0.911813228695652

00:15:02.360 --> 00:15:04.592 it was the number one ranked

NOTE Confidence: 0.911813228695652

00:15:04.592 --> 00:15:06.428 transcription factor that was able

NOTE Confidence: 0.911813228695652

00:15:06.428 --> 00:15:08.516 to regulate the target genes that

NOTE Confidence: 0.911813228695652

00:15:08.516 --> 00:15:09.560 were differentially expressed.

NOTE Confidence: 0.911813228695652

00:15:09.560 --> 00:15:12.733 It was also the number three potential

NOTE Confidence: 0.911813228695652

00:15:12.733 --> 00:15:14.491 regulator of genes that are up

NOTE Confidence: 0.911813228695652

00:15:14.491 --> 00:15:16.129 regulated in the megacaryocyte fate

NOTE Confidence: 0.911813228695652

00:15:16.129 --> 00:15:17.545 specification and amongst those

NOTE Confidence: 0.911813228695652

00:15:17.545 --> 00:15:19.353 is Mipple which is thrombopotin

NOTE Confidence: 0.911813228695652

00:15:19.353 --> 00:15:21.691 receptor and FLEA one which is a

NOTE Confidence: 0.911813228695652

00:15:21.691 --> 00:15:23.960 known transcription factor that's

NOTE Confidence: 0.911813228695652

00:15:23.960 --> 00:15:28.880 critical for megacaryois what we oops,

NOTE Confidence: 0.911813228695652

00:15:28.880 --> 00:15:30.116 this is supposed to come next.

NOTE Confidence: 0.911813228695652

00:15:30.120 --> 00:15:32.346 What we did then was we over

NOTE Confidence: 0.911813228695652

00:15:32.346 --> 00:15:34.447 expressed Runks 1 and when we do

NOTE Confidence: 0.911813228695652

00:15:34.447 --> 00:15:36.607 that you can see that we actually

NOTE Confidence: 0.911813228695652

00:15:36.607 --> 00:15:39.427 caused those bipotent cells to go

NOTE Confidence: 0.911813228695652

00:15:39.427 --> 00:15:41.185 towards the megacaryocyte lineage.

NOTE Confidence: 0.911813228695652

00:15:41.185 --> 00:15:43.600 And then when we inhibited Bronx 1,

NOTE Confidence: 0.911813228695652

00:15:43.600 --> 00:15:46.160 Runks 2 and Runks 3 with a drug,

NOTE Confidence: 0.911813228695652

00:15:46.160 --> 00:15:48.472 we could see the opposite effect where we

NOTE Confidence: 0.911813228695652

00:15:48.472 --> 00:15:50.977 see an increased in E fate specification

NOTE Confidence: 0.911813228695652

00:15:50.977 --> 00:15:53.510 which really proved that the Runcs one

NOTE Confidence: 0.911813228695652

00:15:53.510 --> 00:15:57.640 activity is promoting the MK fate in the MEP.

NOTE Confidence: 0.911813228695652

00:15:57.640 --> 00:15:57.961 However,

NOTE Confidence: 0.911813228695652

00:15:57.961 --> 00:16:00.529 when we looked at Runcs One RNA and

NOTE Confidence: 0.911813228695652

00:16:00.529 --> 00:16:02.836 protein levels in these three lineages,

NOTE Confidence: 0.911813228695652

00:16:02.840 --> 00:16:04.394 the Meg erythroid progenitor and then the

NOTE Confidence: 0.911813228695652

00:16:04.394 --> 00:16:06.200 Meg and the erythroid committed cells,

NOTE Confidence: 0.911813228695652

00:16:06.200 --> 00:16:08.234 there was no difference in either

NOTE Confidence: 0.911813228695652

00:16:08.234 --> 00:16:10.027 protein or RNA expression between

NOTE Confidence: 0.911813228695652

00:16:10.027 --> 00:16:11.922 the Meg committed cells and

NOTE Confidence: 0.911813228695652

00:16:11.922 --> 00:16:13.438 the erythroid committed cells.

NOTE Confidence: 0.911813228695652

00:16:13.440 --> 00:16:15.216 Which told us it's not happening

NOTE Confidence: 0.911813228695652

00:16:15.216 --> 00:16:16.400 at the transcriptional level

NOTE Confidence: 0.911813228695652

00:16:16.449 --> 00:16:17.757 or the translational level.

NOTE Confidence: 0.911813228695652

00:16:17.760 --> 00:16:19.308 It's probably post translational.

NOTE Confidence: 0.911813228695652

00:16:19.308 --> 00:16:21.630 So we started looking at post

NOTE Confidence: 0.911813228695652

00:16:21.698 --> 00:16:24.158 translational modifications of Runx one.

NOTE Confidence: 0.911813228695652

00:16:24.160 --> 00:16:26.106 And one that has been heavily studied

NOTE Confidence: 0.911813228695652

00:16:26.106 --> 00:16:27.720 before is serine and threonine.

NOTE Confidence: 0.911813228695652

00:16:27.720 --> 00:16:30.186 Phosphorylation of Runx one is known

NOTE Confidence: 0.911813228695652

00:16:30.186 --> 00:16:33.398 to be necessary for its activation for

NOTE Confidence: 0.911813228695652

00:16:33.398 --> 00:16:35.878 its ability to activate transgenes.

NOTE Confidence: 0.911813228695652

00:16:35.880 --> 00:16:37.704 So activate transcription.

NOTE Confidence: 0.911813228695652

00:16:37.704 --> 00:16:38.920 I'm sorry.

NOTE Confidence: 0.911813228695652

00:16:38.920 --> 00:16:40.915 What we did is we got antibodies

NOTE Confidence: 0.911813228695652

00:16:40.915 --> 00:16:42.697 that are specific for different

NOTE Confidence: 0.911813228695652

00:16:42.697 --> 00:16:43.996 phosphoserines on Ronks.

NOTE Confidence: 0.911813228695652

00:16:44.000 --> 00:16:45.972 One from our collaborator,

NOTE Confidence: 0.911813228695652

00:16:45.972 --> 00:16:46.958 Alan Friedman,

NOTE Confidence: 0.911813228695652

00:16:46.960 --> 00:16:48.960 he'd published this and pulled them out of

NOTE Confidence: 0.911813228695652

00:16:48.960 --> 00:16:51.040 the freezer for us and they work beautifully.

NOTE Confidence: 0.911813228695652

00:16:51.040 --> 00:16:51.960 And what you can see,

NOTE Confidence: 0.911813228695652

00:16:51.960 --> 00:16:54.093 and this is work that was done by two

NOTE Confidence: 0.911813228695652

00:16:54.093 --> 00:16:56.320 very talented people in the laboratory.

NOTE Confidence: 0.911813228695652

00:16:56.320 --> 00:16:58.330 I already introduced you to Yi

NOTE Confidence: 0.911813228695652

00:16:58.330 --> 00:17:00.610 Shen and Nayeong Kwan is a grad

NOTE Confidence: 0.911813228695652

00:17:00.610 --> 00:17:02.640 student in the lab and she's really

NOTE Confidence: 0.821214104

00:17:02.709 --> 00:17:04.683 been the mastermind between all of

NOTE Confidence: 0.821214104

00:17:04.683 --> 00:17:08.200 all the work I'm about to show you.

NOTE Confidence: 0.821214104

00:17:08.200 --> 00:17:10.699 What she did is she did intracellular

NOTE Confidence: 0.821214104

00:17:10.699 --> 00:17:12.605 flow cytometry for total runks

NOTE Confidence: 0.821214104

00:17:12.605 --> 00:17:14.520 one and phosphoserine runks 1.

NOTE Confidence: 0.821214104

00:17:14.520 --> 00:17:15.682 And I'm going to show you data

NOTE Confidence: 0.821214104

00:17:15.682 --> 00:17:16.920 for several of the phosphoserines.

NOTE Confidence: 0.821214104

00:17:16.920 --> 00:17:18.120 This is phosphoserine 76.

NOTE Confidence: 0.821214104

00:17:18.120 --> 00:17:19.920 I just bother to show you.

NOTE Confidence: 0.821214104

00:17:19.920 --> 00:17:22.664 The 276 is here and phosphoserine 303

NOTE Confidence: 0.821214104

00:17:22.664 --> 00:17:25.838 which is here in the Runks one protein.

NOTE Confidence: 0.821214104

00:17:25.840 --> 00:17:27.984 And what she showed and this is just

NOTE Confidence: 0.821214104

00:17:27.984 --> 00:17:29.453 representative data on the left and

NOTE Confidence: 0.821214104

00:17:29.453 --> 00:17:31.148 then graphed here on the right for

NOTE Confidence: 0.821214104

00:17:31.148 --> 00:17:32.753 multiple replicates is that either

NOTE Confidence: 0.821214104

00:17:32.753 --> 00:17:34.610 with commitment to erythroid or

NOTE Confidence: 0.821214104

00:17:34.610 --> 00:17:36.320 megacarocyte fate specification,

NOTE Confidence: 0.821214104

00:17:36.320 --> 00:17:40.759 you see an increase in the phosphosurine

NOTE Confidence: 0.821214104

00:17:40.759 --> 00:17:44.074 levels of Bronx One and there's a

NOTE Confidence: 0.821214104

00:17:44.074 --> 00:17:45.670 significantly higher increase when

NOTE Confidence: 0.821214104

00:17:45.738 --> 00:17:48.160 you go to the Meg Fate specification.

NOTE Confidence: 0.821214104

00:17:48.160 --> 00:17:51.420 Similarly with the Erythroid, Similarly,

NOTE Confidence: 0.821214104

00:17:51.420 --> 00:17:53.320 I'm sorry, with phosphosurine 303,

NOTE Confidence: 0.821214104

00:17:53.320 --> 00:17:56.312 you see this increase and then a further

NOTE Confidence: 0.821214104

00:17:56.312 --> 00:17:58.151 significant increase between erythroid

NOTE Confidence: 0.821214104

00:17:58.151 --> 00:18:00.195 progenitors and Meg progenitors.

NOTE Confidence: 0.821214104

00:18:00.200 --> 00:18:01.718 So that's just shown schematically here.

NOTE Confidence: 0.821214104

00:18:01.720 --> 00:18:04.478 The phosphoserine runcs 1 levels go up

NOTE Confidence: 0.821214104

00:18:04.478 --> 00:18:07.635 from MEP to MKP and go down or don't

NOTE Confidence: 0.821214104

00:18:07.635 --> 00:18:10.557 go up with when you go to Erythroid.

NOTE Confidence: 0.821214104

00:18:10.560 --> 00:18:12.240 So this is where we are.

NOTE Confidence: 0.821214104

00:18:12.240 --> 00:18:14.424 Is there a link now with the cell

NOTE Confidence: 0.821214104

00:18:14.424 --> 00:18:16.160 cycle data that I showed you?

NOTE Confidence: 0.821214104

00:18:16.160 --> 00:18:17.648 I'll just show you one example

NOTE Confidence: 0.821214104

00:18:17.648 --> 00:18:18.640 to for this link.

NOTE Confidence: 0.821214104

00:18:18.640 --> 00:18:21.440 The link is basically that the slowing

NOTE Confidence: 0.821214104

00:18:21.440 --> 00:18:24.560 of the cell cycle requires Runcs 1.

NOTE Confidence: 0.821214104

00:18:24.560 --> 00:18:26.288 You if you slow the cell cycle and

NOTE Confidence: 0.821214104

00:18:26.288 --> 00:18:27.943 there's no Runx One activity then you

NOTE Confidence: 0.821214104

00:18:27.943 --> 00:18:29.840 don't get the Meg Fate specification.

NOTE Confidence: 0.821214104

00:18:29.840 --> 00:18:31.639 They still go down the erythroid lineage.

NOTE Confidence: 0.821214104

00:18:31.640 --> 00:18:33.320 But let me show that to you slowly.

NOTE Confidence: 0.821214104

00:18:33.320 --> 00:18:35.357 So here's your control with the bipotent,

NOTE Confidence: 0.821214104

00:18:35.360 --> 00:18:37.320 the erythroid only and the Meg only.

NOTE Confidence: 0.821214104

00:18:37.320 --> 00:18:39.561 This is the effect of the Runx 1 inhibitor

NOTE Confidence: 0.821214104

00:18:39.561 --> 00:18:41.398 that gives us more erythroid only.

NOTE Confidence: 0.821214104

00:18:41.400 --> 00:18:43.101 This is the effect I showed you

NOTE Confidence: 0.821214104

00:18:43.101 --> 00:18:44.372 previously of ATRA or rapamycin

NOTE Confidence: 0.821214104

00:18:44.372 --> 00:18:46.101 where they slow the cell cycle and

NOTE Confidence: 0.821214104

00:18:46.101 --> 00:18:47.960 you get more Meg Fate specification.

NOTE Confidence: 0.821214104

00:18:47.960 --> 00:18:50.438 And here I'm showing you the combination.

NOTE Confidence: 0.821214104

00:18:50.440 --> 00:18:52.820 You do not see this increase in

NOTE Confidence: 0.821214104

00:18:52.820 --> 00:18:54.527 Meg phase specification with ATRA

NOTE Confidence: 0.821214104

00:18:54.527 --> 00:18:56.381 or with rapamycin in the presence

NOTE Confidence: 0.821214104

00:18:56.381 --> 00:18:58.679 of the inhibitor of the Ronx one.

NOTE Confidence: 0.821214104

00:18:58.680 --> 00:19:01.025 Really suggesting that we have a link

NOTE Confidence: 0.821214104

00:19:01.025 --> 00:19:03.573 now between slowing the cell cycle and

NOTE Confidence: 0.821214104

00:19:03.573 --> 00:19:05.423 getting increased Ronx 1 phosphorylation

NOTE Confidence: 0.821214104

00:19:05.423 --> 00:19:07.798 and increased MK phase specification.

NOTE Confidence: 0.70433965

00:19:11.600 --> 00:19:12.638 Oh, so I did include this,

NOTE Confidence: 0.70433965

00:19:12.640 --> 00:19:13.599 I wasn't sure if I'd show this.

NOTE Confidence: 0.70433965

00:19:13.600 --> 00:19:15.224 So then we actually did prove that

NOTE Confidence: 0.70433965

00:19:15.224 --> 00:19:17.207 if you slow the cell cycle like

NOTE Confidence: 0.70433965

00:19:17.207 --> 00:19:18.747 with the Pelvicyclib that actually

NOTE Confidence: 0.70433965

00:19:18.747 --> 00:19:20.192 slowed the cell cycle and then

NOTE Confidence: 0.70433965

00:19:20.192 --> 00:19:22.132 you wash it out and then you show

NOTE Confidence: 0.70433965

00:19:22.132 --> 00:19:23.836 the cells are Meg fate specified,

NOTE Confidence: 0.70433965

00:19:23.840 --> 00:19:26.594 you actually get increased levels of

NOTE Confidence: 0.70433965

00:19:26.594 --> 00:19:29.607 phosphosurine runks one at both 276 and 303.

NOTE Confidence: 0.70433965

00:19:29.607 --> 00:19:31.490 It ends up you also get increased

NOTE Confidence: 0.70433965

00:19:31.556 --> 00:19:33.076 levels of total runks one,

NOTE Confidence: 0.70433965

00:19:33.080 --> 00:19:35.432 but the ratios suggest that we probably

NOTE Confidence: 0.70433965

00:19:35.432 --> 00:19:37.775 have a higher percentage of the

NOTE Confidence: 0.70433965

00:19:37.775 --> 00:19:39.880 total runks that is phosphorylated.

NOTE Confidence: 0.70433965

00:19:39.880 --> 00:19:43.136 So that's our link for now with fate

NOTE Confidence: 0.70433965

00:19:43.136 --> 00:19:45.160 specification and the cell cycle.

NOTE Confidence: 0.70433965

00:19:45.160 --> 00:19:47.386 We then wanted to test the effects

NOTE Confidence: 0.70433965

00:19:47.386 --> 00:19:49.899 on primary cells if we get rid of

NOTE Confidence: 0.70433965

00:19:49.899 --> 00:19:51.394 the serines and threonines that

NOTE Confidence: 0.70433965

00:19:51.461 --> 00:19:53.519 are phosphorylated in the Runx 1,

NOTE Confidence: 0.70433965

00:19:53.520 --> 00:19:54.560 So I'd shown you previously,

NOTE Confidence: 0.70433965

00:19:54.560 --> 00:19:55.880 when we overexpressed Runx one,

NOTE Confidence: 0.70433965

00:19:55.880 --> 00:19:58.196 we get more MK fate specification.

NOTE Confidence: 0.70433965

00:19:58.200 --> 00:20:01.196 What if we mutate these four residues,

NOTE Confidence: 0.70433965

00:20:01.200 --> 00:20:03.520 3 serines and one threonine

NOTE Confidence: 0.70433965

00:20:03.520 --> 00:20:05.840 to alanine in that case?

NOTE Confidence: 0.70433965

00:20:05.840 --> 00:20:08.078 We didn't get quite no effect.

NOTE Confidence: 0.70433965

00:20:08.080 --> 00:20:08.772 We got some effect,

NOTE Confidence: 0.70433965

00:20:08.772 --> 00:20:10.046 but it was a less strong effect

NOTE Confidence: 0.70433965

00:20:10.046 --> 00:20:10.996 than in the wild type.

NOTE Confidence: 0.70433965

00:20:11.000 --> 00:20:13.555 And in contrast when we changed the

NOTE Confidence: 0.70433965

00:20:13.555 --> 00:20:15.485 serines and threonines to aspartic

NOTE Confidence: 0.70433965

00:20:15.485 --> 00:20:17.795 acid which mimics the phospho serine,

NOTE Confidence: 0.70433965

00:20:17.800 --> 00:20:19.886 so all of the overexpressed brunx one

NOTE Confidence: 0.70433965

00:20:19.886 --> 00:20:22.558 is pre in a pre phosphorylated state.

NOTE Confidence: 0.70433965

00:20:22.560 --> 00:20:25.283 We got a far stronger effect with

NOTE Confidence: 0.70433965

00:20:25.283 --> 00:20:27.248 almost no erythroid fate specification

NOTE Confidence: 0.70433965

00:20:27.248 --> 00:20:29.710 and a lot of MK only suggesting

NOTE Confidence: 0.70433965

00:20:29.710 --> 00:20:31.570 that this is really playing a

NOTE Confidence: 0.70433965

00:20:31.570 --> 00:20:33.720 role in MK fate specification.

NOTE Confidence: 0.70433965

00:20:33.720 --> 00:20:35.172 In order to study this we

NOTE Confidence: 0.70433965

00:20:35.172 --> 00:20:36.840 then used a cell line model.

NOTE Confidence: 0.70433965

00:20:36.840 --> 00:20:38.600 So human erythro leukemia

NOTE Confidence: 0.70433965

00:20:38.600 --> 00:20:40.521 cells are an OK model.

NOTE Confidence: 0.70433965

00:20:40.521 --> 00:20:42.256 When you add TPA they

NOTE Confidence: 0.70433965

00:20:42.256 --> 00:20:44.360 go down the Meg lineage,

NOTE Confidence: 0.70433965

00:20:44.360 --> 00:20:45.872 when you add hemen they kind of sort

NOTE Confidence: 0.70433965

00:20:45.872 --> 00:20:47.517 of go down the erythroid lineage.

NOTE Confidence: 0.70433965

00:20:47.520 --> 00:20:49.298 Anyway it's the best system we have

NOTE Confidence: 0.70433965

00:20:49.298 --> 00:20:51.251 for looking at this and what we wanted

NOTE Confidence: 0.70433965

00:20:51.251 --> 00:20:53.160 to do is over express the wild type,

NOTE Confidence: 0.70433965

00:20:53.160 --> 00:20:55.600 the 4A mutant that has the alanine mutations,

NOTE Confidence: 0.70433965

00:20:55.600 --> 00:20:57.693 the 4D mutant with the aspartic acid

NOTE Confidence: 0.70433965

00:20:57.693 --> 00:21:00.059 mutations and so that we can do some

NOTE Confidence: 0.70433965

00:21:00.059 --> 00:21:01.866 molecular studies like cut and run

NOTE Confidence: 0.70433965

00:21:01.866 --> 00:21:03.556 and and gene expression changes.

NOTE Confidence: 0.70433965

00:21:03.560 --> 00:21:06.080 And first thing you can see is even

NOTE Confidence: 0.70433965

00:21:06.080 --> 00:21:07.716 without inducing these cells to

NOTE Confidence: 0.70433965

00:21:07.716 --> 00:21:09.606 differentiate with TPA we just we

NOTE Confidence: 0.70433965

00:21:09.606 --> 00:21:11.920 get them to go down the Meg lineage.

NOTE Confidence: 0.70433965

00:21:11.920 --> 00:21:14.840 CD 42 comes on in more mature megakaryocytes.

NOTE Confidence: 0.70433965

00:21:14.840 --> 00:21:16.835 You can see that they already start

NOTE Confidence: 0.70433965

00:21:16.835 --> 00:21:18.800 to mature down the Meg lineage

NOTE Confidence: 0.70433965

00:21:18.800 --> 00:21:20.965 just by over expressing this pre

NOTE Confidence: 0.70433965

00:21:20.965 --> 00:21:22.905 phosphorylated Bronx one compared

NOTE Confidence: 0.70433965

00:21:22.905 --> 00:21:26.400 to the 4A or the wild type.

NOTE Confidence: 0.70433965

00:21:26.400 --> 00:21:28.010 We then looked at gene expression changes

NOTE Confidence: 0.70433965

00:21:28.010 --> 00:21:29.879 and cut and run in these health cells.

NOTE Confidence: 0.70433965

00:21:29.880 --> 00:21:31.518 I'm just showing you gene expression changes.

NOTE Confidence: 0.70433965

00:21:31.520 --> 00:21:33.350 First glycoprotein 1B beta is a

NOTE Confidence: 0.70433965

00:21:33.350 --> 00:21:35.121 gene that's very important in Meg

NOTE Confidence: 0.70433965

00:21:35.121 --> 00:21:36.603 maturation and what you can see

NOTE Confidence: 0.70433965

00:21:36.603 --> 00:21:38.494 and this is just two duplicates of

NOTE Confidence: 0.70433965

00:21:38.494 --> 00:21:40.200 each for the empty vector cells.

NOTE Confidence: 0.70433965

00:21:40.200 --> 00:21:42.360 For the cells that we're expressing

NOTE Confidence: 0.70433965

00:21:42.360 --> 00:21:43.870 4A that cannot be phosphorylated

NOTE Confidence: 0.70433965

00:21:43.870 --> 00:21:45.078 on this four residues,

NOTE Confidence: 0.70433965

00:21:45.080 --> 00:21:47.565 the wild type and the 4D that's

NOTE Confidence: 0.70433965

00:21:47.565 --> 00:21:48.275 pre phosphorylated.

NOTE Confidence: 0.70433965

00:21:48.280 --> 00:21:49.966 And what you can see is

NOTE Confidence: 0.70433965

00:21:49.966 --> 00:21:51.090 this gradual increase in

NOTE Confidence: 0.891751959166667

00:21:51.158 --> 00:21:53.270 the glycoprotein 1B beta consistent with

NOTE Confidence: 0.891751959166667

00:21:53.270 --> 00:21:55.932 the increased CD 42 that we had seen when

NOTE Confidence: 0.891751959166667

00:21:55.932 --> 00:21:58.200 we looked at where is that Runx one bound.

NOTE Confidence: 0.891751959166667

00:21:58.200 --> 00:22:00.223 So the over expressed 4A4D and wild

NOTE Confidence: 0.891751959166667

00:22:00.223 --> 00:22:02.390 tape are all HA tagged and when we

NOTE Confidence: 0.891751959166667

00:22:02.390 --> 00:22:04.659 did anti HA cut and run what we

NOTE Confidence: 0.891751959166667

00:22:04.659 --> 00:22:06.279 found is there's no difference,

NOTE Confidence: 0.891751959166667

00:22:06.280 --> 00:22:07.920 they all bind just fine.

NOTE Confidence: 0.891751959166667

00:22:07.920 --> 00:22:10.524 This isn't a complete surprise because

NOTE Confidence: 0.891751959166667

00:22:10.524 --> 00:22:12.946 the DNA binding domain of Runx One

NOTE Confidence: 0.891751959166667

00:22:12.946 --> 00:22:15.240 is not near those phosphocytes.

NOTE Confidence: 0.891751959166667

00:22:15.240 --> 00:22:17.094 But what it strongly suggests is

NOTE Confidence: 0.891751959166667

00:22:17.094 --> 00:22:20.083 that Runx one can bind but the post

NOTE Confidence: 0.891751959166667

00:22:20.083 --> 00:22:21.767 translational modification is what's

NOTE Confidence: 0.891751959166667

00:22:21.767 --> 00:22:24.158 affecting its effect on transcription.

NOTE Confidence: 0.891751959166667

00:22:24.160 --> 00:22:26.272 And keep that in mind because I think

NOTE Confidence: 0.891751959166667

00:22:26.272 --> 00:22:28.566 we we start to have clues now as

NOTE Confidence: 0.891751959166667

00:22:28.566 --> 00:22:30.558 to where that might be taking us.

NOTE Confidence: 0.891751959166667

00:22:30.560 --> 00:22:32.387 This is just showing you that when

NOTE Confidence: 0.891751959166667

00:22:32.387 --> 00:22:34.326 we combine the cut and run and the

NOTE Confidence: 0.891751959166667

00:22:34.326 --> 00:22:36.291 RNA seek data that we have this group

NOTE Confidence: 0.891751959166667

00:22:36.291 --> 00:22:38.195 of genes that are activated by both

NOTE Confidence: 0.891751959166667

00:22:38.200 --> 00:22:40.704 wild type and 4D in the health cells

NOTE Confidence: 0.891751959166667

00:22:40.704 --> 00:22:42.976 but not as much by the 4A mutant.

NOTE Confidence: 0.891751959166667

00:22:42.976 --> 00:22:43.552 But yeah,

NOTE Confidence: 0.891751959166667

00:22:43.552 --> 00:22:45.615 the 4A mutant and those genes tend

NOTE Confidence: 0.891751959166667

00:22:45.615 --> 00:22:47.823 to be genes that we know are very

NOTE Confidence: 0.891751959166667

00:22:47.823 --> 00:22:49.198 important in Meg maturation.

NOTE Confidence: 0.891751959166667

00:22:49.200 --> 00:22:50.892 So just consistent with what I

NOTE Confidence: 0.891751959166667

00:22:50.892 --> 00:22:52.838 already showed you on the other two,

NOTE Confidence: 0.891751959166667

00:22:52.840 --> 00:22:54.240 I'm not going to show you a whole

NOTE Confidence: 0.891751959166667

00:22:54.240 --> 00:22:55.657 lot of data and a whole lot of

NOTE Confidence: 0.891751959166667

00:22:55.657 --> 00:22:56.960 work on the cut and run data,

NOTE Confidence: 0.891751959166667

00:22:56.960 --> 00:23:00.120 except to say that there really was no

NOTE Confidence: 0.891751959166667

00:23:00.120 --> 00:23:01.777 significant difference in binding of

NOTE Confidence: 0.891751959166667

00:23:01.777 --> 00:23:04.160 the four a the wild type in the 4D.

NOTE Confidence: 0.891751959166667

00:23:04.160 --> 00:23:06.197 So the next question is what phosphorylates

NOTE Confidence: 0.891751959166667

00:23:06.197 --> 00:23:08.037 the runks one and this has been,

NOTE Confidence: 0.891751959166667

00:23:08.040 --> 00:23:09.000 this is very recent data,

NOTE Confidence: 0.891751959166667

00:23:09.000 --> 00:23:10.056 it's not yet published.

NOTE Confidence: 0.891751959166667

00:23:10.056 --> 00:23:11.640 A lot of this isn't published,

NOTE Confidence: 0.891751959166667

00:23:11.640 --> 00:23:13.440 but this is like we got it in

NOTE Confidence: 0.891751959166667

00:23:13.440 --> 00:23:14.599 the last few months.

NOTE Confidence: 0.891751959166667

00:23:14.600 --> 00:23:16.660 Multiple kinases were published

NOTE Confidence: 0.891751959166667

00:23:16.660 --> 00:23:18.720 that phosphorylate runks one,

NOTE Confidence: 0.891751959166667

00:23:18.720 --> 00:23:20.390 and problem is whether when

NOTE Confidence: 0.891751959166667

00:23:20.390 --> 00:23:22.480 we knock down any of them,

NOTE Confidence: 0.891751959166667

00:23:22.480 --> 00:23:27.439 we had no loss of phosphorylation on Runx 1.

NOTE Confidence: 0.891751959166667

00:23:27.440 --> 00:23:28.464 So what's going on?

NOTE Confidence: 0.891751959166667

00:23:28.464 --> 00:23:30.920 We decided what has to be another kinase,

NOTE Confidence: 0.891751959166667

00:23:30.920 --> 00:23:32.404 so I'm going to take you through

NOTE Confidence: 0.891751959166667

00:23:32.404 --> 00:23:33.280 that a little bit.

NOTE Confidence: 0.891751959166667

00:23:33.280 --> 00:23:36.380 The predicted kinases for Runx

NOTE Confidence: 0.891751959166667

00:23:36.380 --> 00:23:38.240 one include CD,

NOTE Confidence: 0.891751959166667

00:23:38.240 --> 00:23:39.680 all of the cycling dependent kinases,

NOTE Confidence: 0.891751959166667

00:23:39.680 --> 00:23:41.992 and CDKS 1-2 and six had all been

NOTE Confidence: 0.891751959166667

00:23:41.992 --> 00:23:44.399 proven to phosphorylate it in vitro.

NOTE Confidence: 0.891751959166667

00:23:44.400 --> 00:23:45.640 Similarly with the SIP,

NOTE Confidence: 0.891751959166667

00:23:45.640 --> 00:23:46.880 K2 and the URC.

NOTE Confidence: 0.891751959166667

00:23:46.880 --> 00:23:48.427 But all of their activity was shown

NOTE Confidence: 0.891751959166667

00:23:48.427 --> 00:23:49.965 in reporter assays and it didn't end

NOTE Confidence: 0.891751959166667

00:23:49.965 --> 00:23:51.213 up being relevant for our primary

NOTE Confidence: 0.891751959166667

00:23:51.263 --> 00:23:52.488 cells where the phospho levels

NOTE Confidence: 0.891751959166667

00:23:52.488 --> 00:23:54.091 didn't change when we knocked down

NOTE Confidence: 0.891751959166667

00:23:54.091 --> 00:23:56.317 these genes or inhibited them with

NOTE Confidence: 0.891751959166667

00:23:56.320 --> 00:23:59.680 with with very small molecules.

NOTE Confidence: 0.891751959166667

00:23:59.680 --> 00:24:00.388 In fact,

NOTE Confidence: 0.891751959166667

00:24:00.388 --> 00:24:02.158 if you what we found

NOTE Confidence: 0.714086705

00:24:02.160 --> 00:24:03.040 is if if you

NOTE Confidence: 0.90280320625

00:24:04.200 --> 00:24:05.610 inhibit CDK 9,

NOTE Confidence: 0.90280320625

00:24:05.610 --> 00:24:07.960 which is completely different CDK,

NOTE Confidence: 0.90280320625

00:24:07.960 --> 00:24:09.040 that's when you lose it.

NOTE Confidence: 0.90280320625

00:24:09.040 --> 00:24:10.160 So I'm going to show you first,

NOTE Confidence: 0.90280320625

00:24:10.160 --> 00:24:11.280 this is what happens when

NOTE Confidence: 0.90280320625

00:24:11.280 --> 00:24:12.640 we inhibit CDK four or six.

NOTE Confidence: 0.90280320625

00:24:12.640 --> 00:24:14.116 So they were predicted.

NOTE Confidence: 0.90280320625

00:24:14.116 --> 00:24:15.961 CDK six was predicted to

NOTE Confidence: 0.90280320625

00:24:15.961 --> 00:24:17.996 be a kinase for Runx One.

NOTE Confidence: 0.90280320625

00:24:18.000 --> 00:24:19.460 I previously showed you these

NOTE Confidence: 0.90280320625

00:24:19.460 --> 00:24:20.920 data in a different context.

NOTE Confidence: 0.90280320625

00:24:20.920 --> 00:24:22.439 When you inhibit CDK four and six,

NOTE Confidence: 0.90280320625

00:24:22.440 --> 00:24:23.820 you actually get more

NOTE Confidence: 0.90280320625

00:24:23.820 --> 00:24:25.200 phosphorylation of Runx One.

NOTE Confidence: 0.90280320625

00:24:25.200 --> 00:24:26.748 Remember that was consistent with slowing

NOTE Confidence: 0.90280320625

00:24:26.748 --> 00:24:28.798 the cell cycle more Runx 1 phosphorylation.

NOTE Confidence: 0.90280320625

00:24:28.800 --> 00:24:31.640 So CDK 6 is not the thing that's

NOTE Confidence: 0.90280320625

00:24:31.640 --> 00:24:33.560 phosphorylating Runx One in our cells.

NOTE Confidence: 0.90280320625

00:24:33.560 --> 00:24:36.560 But when we inhibited CDK 9,

NOTE Confidence: 0.90280320625

00:24:36.560 --> 00:24:38.340 which was another predicted kinase

NOTE Confidence: 0.90280320625

00:24:38.340 --> 00:24:40.120 that would phosphorylate these cells,

NOTE Confidence: 0.90280320625

00:24:40.120 --> 00:24:41.878 then we saw something really interesting.

NOTE Confidence: 0.90280320625

00:24:41.880 --> 00:24:43.692 Then the total level of Bronx

NOTE Confidence: 0.90280320625

00:24:43.692 --> 00:24:44.598 One didn't change,

NOTE Confidence: 0.90280320625

00:24:44.600 --> 00:24:46.784 but the levels of both phosphoserine

NOTE Confidence: 0.90280320625

00:24:46.784 --> 00:24:49.068 3O3 and phosphoserine 276 did change.

NOTE Confidence: 0.90280320625

00:24:49.068 --> 00:24:51.840 Now this is one of several flavopyridol

NOTE Confidence: 0.90280320625

00:24:51.917 --> 00:24:54.437 is one of several CDK 9 inhibitors,

NOTE Confidence: 0.90280320625

00:24:54.440 --> 00:24:56.648 but none of them is absolutely

NOTE Confidence: 0.90280320625

00:24:56.648 --> 00:24:58.120 specific for CDK 9:00.

NOTE Confidence: 0.90280320625

00:24:58.120 --> 00:24:59.975 So we ended up getting a different

NOTE Confidence: 0.90280320625

00:24:59.975 --> 00:25:01.999 CDK 9 inhibitor that is more specific.

NOTE Confidence: 0.90280320625

00:25:02.000 --> 00:25:04.238 It's called phallus NSO 3 two,

NOTE Confidence: 0.90280320625

00:25:04.240 --> 00:25:07.117 and it induces degradation of CDK 9,

NOTE Confidence: 0.90280320625

00:25:07.120 --> 00:25:09.024 which I'm not showing you, but it does.

NOTE Confidence: 0.90280320625

00:25:09.024 --> 00:25:11.232 And when we added the Thou,

NOTE Confidence: 0.90280320625

00:25:11.240 --> 00:25:13.718 we also got the loss of the

NOTE Confidence: 0.90280320625

00:25:13.718 --> 00:25:16.088 phosphoserine 3O3 and phosphoserine 276.

NOTE Confidence: 0.90280320625

00:25:16.088 --> 00:25:18.680 And when we added the Thou to the cells,

NOTE Confidence: 0.90280320625

00:25:18.680 --> 00:25:19.760 just as we had expected,

NOTE Confidence: 0.90280320625

00:25:19.760 --> 00:25:22.960 we got an erythroid bias to our MEP.

NOTE Confidence: 0.90280320625

00:25:22.960 --> 00:25:23.646 Really.

NOTE Confidence: 0.90280320625

00:25:23.646 --> 00:25:29.134 Now connecting CDK 9 activity to Ronx 1

NOTE Confidence: 0.90280320625

00:25:29.134 --> 00:25:32.757 phosphorylation to MEP Fate specification.

NOTE Confidence: 0.90280320625

00:25:32.760 --> 00:25:34.880 Now for those of you who know what CDK 9 is,

NOTE Confidence: 0.90280320625

00:25:34.880 --> 00:25:35.768 this is just like,

NOTE Confidence: 0.90280320625

00:25:35.768 --> 00:25:36.902 Oh my God, what's it doing?

NOTE Confidence: 0.90280320625

00:25:36.902 --> 00:25:38.200 And I the answer is I don't know.

NOTE Confidence: 0.90280320625

00:25:38.200 --> 00:25:40.360 But for those of you who

NOTE Confidence: 0.90280320625

00:25:40.360 --> 00:25:41.320 don't know what CDK 9 is,

NOTE Confidence: 0.90280320625

00:25:41.320 --> 00:25:42.790 the reason this is exciting is

NOTE Confidence: 0.90280320625

00:25:42.790 --> 00:25:45.613 CDK 9 is part of just the general

NOTE Confidence: 0.90280320625

00:25:45.613 --> 00:25:48.520 transcriptional control apparatus.

NOTE Confidence: 0.90280320625

00:25:48.520 --> 00:25:52.440 It's part of activating RNA polymerase too,

NOTE Confidence: 0.90280320625

00:25:52.440 --> 00:25:54.408 but in published data from years

NOTE Confidence: 0.90280320625

00:25:54.408 --> 00:25:56.759 ago that has never been explained.

NOTE Confidence: 0.90280320625

00:25:56.760 --> 00:26:00.468 Knock down the CDK 9 causes you to lose

NOTE Confidence: 0.90280320625

00:26:00.468 --> 00:26:02.400 megacary sites and people never knew why.

NOTE Confidence: 0.90280320625

00:26:02.400 --> 00:26:04.840 So I think we now have a link between CDK 9,

NOTE Confidence: 0.90280320625

00:26:04.840 --> 00:26:07.114 Runx One and Meg Fate specification

NOTE Confidence: 0.90280320625

00:26:07.114 --> 00:26:09.998 that we have a grant to look at.

NOTE Confidence: 0.90280320625

00:26:10.000 --> 00:26:13.262 So the summary of Part 2 is that

NOTE Confidence: 0.90280320625

00:26:13.262 --> 00:26:15.717 phosphosurine RUNX 1 promotes Meg

NOTE Confidence: 0.90280320625

00:26:15.717 --> 00:26:17.659 Fate specification that's through

NOTE Confidence: 0.90280320625

00:26:17.659 --> 00:26:20.771 phosphorylation by CDK 9 which is part of

NOTE Confidence: 0.90280320625

00:26:20.771 --> 00:26:22.348 the transcriptional regulatory complex.

NOTE Confidence: 0.90280320625

00:26:22.348 --> 00:26:25.456 And the work that we're in the process

NOTE Confidence: 0.90280320625

00:26:25.456 --> 00:26:27.436 of doing that I I don't know the

NOTE Confidence: 0.90280320625

00:26:27.436 --> 00:26:29.537 answer to it yet is what is their

NOTE Confidence: 0.90280320625

00:26:29.537 --> 00:26:30.993 differential binding as phosphosirring

NOTE Confidence: 0.90280320625

00:26:30.993 --> 00:26:33.359 runks 1 to different target proteins.

NOTE Confidence: 0.90280320625

00:26:33.360 --> 00:26:35.268 And we really want to do RNA seek and

NOTE Confidence: 0.90280320625

00:26:35.268 --> 00:26:37.302 cut and run on these various different

NOTE Confidence: 0.90280320625

00:26:37.302 --> 00:26:39.190 runks mutants in primary cells because

NOTE Confidence: 0.90280320625

00:26:39.190 --> 00:26:41.082 everything I showed you for that

NOTE Confidence: 0.90280320625

00:26:41.082 --> 00:26:42.797 so far was done in health cells.

NOTE Confidence: 0.90280320625

00:26:42.800 --> 00:26:43.694 And then really,

NOTE Confidence: 0.90280320625

00:26:43.694 --> 00:26:47.003 how does the CDK 9 Pol 2 Runks 1

NOTE Confidence: 0.90280320625

00:26:47.003 --> 00:26:48.725 regulate transcriptional elongation

NOTE Confidence: 0.90280320625

00:26:48.725 --> 00:26:51.595 to promote Meg BAKED specification.

NOTE Confidence: 0.90280320625

00:26:51.600 --> 00:26:52.464 OK Act 3.

NOTE Confidence: 0.90280320625

00:26:52.464 --> 00:26:54.480 So act three is I showed you

NOTE Confidence: 0.914371405555556

00:26:54.565 --> 00:26:57.675 that we get colonies and what we do is we

NOTE Confidence: 0.914371405555556

00:26:57.759 --> 00:27:00.797 read those colonies out after two weeks.

NOTE Confidence: 0.914371405555556

00:27:00.800 --> 00:27:02.360 So you put cells in two weeks later

NOTE Confidence: 0.914371405555556

00:27:02.360 --> 00:27:04.115 you say what colony types do we have,

NOTE Confidence: 0.914371405555556

00:27:04.120 --> 00:27:06.682 but we really then are not not,

NOTE Confidence: 0.914371405555556

00:27:06.682 --> 00:27:08.374 don't know for sure what's happening

NOTE Confidence: 0.914371405555556

00:27:08.374 --> 00:27:10.160 with all the cells in between.

NOTE Confidence: 0.914371405555556

00:27:10.160 --> 00:27:12.242 For example, is there more rapid

NOTE Confidence: 0.914371405555556

00:27:12.242 --> 00:27:14.476 proliferation in the cells before they

NOTE Confidence: 0.914371405555556

00:27:14.476 --> 00:27:16.840 pick the erythroid fate and slower

NOTE Confidence: 0.914371405555556

00:27:16.840 --> 00:27:19.037 proliferation before they pick the Meg fate?

NOTE Confidence: 0.914371405555556

00:27:19.040 --> 00:27:20.160 How are we going to look at that?

NOTE Confidence: 0.914371405555556

00:27:20.160 --> 00:27:23.160 We have to actually watch them

NOTE Confidence: 0.914371405555556

00:27:23.160 --> 00:27:25.160 undergoing this fate specification.

NOTE Confidence: 0.914371405555556

00:27:25.160 --> 00:27:27.592 So what Vanessa Scanlon lab and did in

NOTE Confidence: 0.914371405555556

00:27:27.592 --> 00:27:30.238 my lab and Vanessa has now moved on.

NOTE Confidence: 0.914371405555556

00:27:30.240 --> 00:27:31.878 She was an amazing post doc and

NOTE Confidence: 0.914371405555556

00:27:31.878 --> 00:27:33.918 she's now an assistant professor at

NOTE Confidence: 0.914371405555556

00:27:33.918 --> 00:27:35.838 University of Connecticut and what

NOTE Confidence: 0.914371405555556

00:27:35.838 --> 00:27:38.390 she did is she developed a time lapse

NOTE Confidence: 0.914371405555556

00:27:38.460 --> 00:27:41.370 microscopy to watch individual human MEP

NOTE Confidence: 0.914371405555556

00:27:41.370 --> 00:27:43.800 undergo fate specification in vitro.

NOTE Confidence: 0.91749533

00:27:46.920 --> 00:27:48.360 So here's what she did.

NOTE Confidence: 0.91749533

00:27:48.360 --> 00:27:49.836 She took her facts, sorted MEP.

NOTE Confidence: 0.91749533

00:27:49.840 --> 00:27:51.624 She put very few of them in a

NOTE Confidence: 0.91749533

00:27:51.624 --> 00:27:53.601 very small volume in a in a plate

NOTE Confidence: 0.91749533

00:27:53.601 --> 00:27:55.398 covered that and that in the same

NOTE Confidence: 0.91749533

00:27:55.398 --> 00:27:56.904 semi solid medium that we use

NOTE Confidence: 0.91749533

00:27:56.904 --> 00:27:58.264 for our colony forming essays.

NOTE Confidence: 0.91749533

00:27:58.264 --> 00:27:59.920 But it has it's very flat.

NOTE Confidence: 0.91749533

00:27:59.920 --> 00:28:01.656 She puts a cover slip on top of

NOTE Confidence: 0.91749533

00:28:01.656 --> 00:28:03.316 that puts it into the Viva view.

NOTE Confidence: 0.91749533

00:28:03.320 --> 00:28:04.840 This is an Olympus apparatus

NOTE Confidence: 0.91749533

00:28:04.840 --> 00:28:06.680 we still have in the lab.

NOTE Confidence: 0.91749533

00:28:06.680 --> 00:28:07.580 It works beautifully.

NOTE Confidence: 0.91749533

00:28:07.580 --> 00:28:09.080 They don't make it anymore.

NOTE Confidence: 0.91749533

00:28:09.080 --> 00:28:10.916 So for now we have it and then she

NOTE Confidence: 0.91749533

00:28:10.916 --> 00:28:12.681 can watch these cells undergoing

NOTE Confidence: 0.91749533

00:28:12.681 --> 00:28:14.561 fate specification and add the

NOTE Confidence: 0.91749533

00:28:14.561 --> 00:28:16.253 antibodies towards the end of making

NOTE Confidence: 0.91749533

00:28:16.253 --> 00:28:18.140 the movie so that the erythroid cells

NOTE Confidence: 0.91749533

00:28:18.140 --> 00:28:20.310 under are showing in red and the

NOTE Confidence: 0.91749533

00:28:20.310 --> 00:28:22.116 megacuria sites are showing in green.

NOTE Confidence: 0.91749533

00:28:22.120 --> 00:28:24.514 So here you have a bipotent colony,

NOTE Confidence: 0.91749533

00:28:24.520 --> 00:28:26.440 a mega only colony and an erythroid colony.

NOTE Confidence: 0.91749533

00:28:26.440 --> 00:28:28.156 But they're all very flat because

NOTE Confidence: 0.91749533

00:28:28.156 --> 00:28:30.043 we're looking at this and we're going

NOTE Confidence: 0.91749533

00:28:30.043 --> 00:28:31.960 to want to look at this over time.

NOTE Confidence: 0.91749533

00:28:31.960 --> 00:28:34.137 Here's an example of an MEP colony

NOTE Confidence: 0.91749533

00:28:34.137 --> 00:28:37.091 of an MEP ending up making a mega

NOTE Confidence: 0.91749533

00:28:37.091 --> 00:28:39.036 carry site in erythroid colony.

NOTE Confidence: 0.91749533

00:28:39.040 --> 00:28:40.918 The little dots that color them,

NOTE Confidence: 0.91749533

00:28:40.920 --> 00:28:43.080 we put those in, that's part of our analysis.

NOTE Confidence: 0.91749533

00:28:43.080 --> 00:28:45.660 So I don't have that pre dotted.

NOTE Confidence: 0.91749533

00:28:45.660 --> 00:28:47.480 But anyway, so that's a single cell.

NOTE Confidence: 0.91749533

00:28:47.480 --> 00:28:49.316 We're starting with a single MEP

NOTE Confidence: 0.91749533

00:28:49.320 --> 00:28:50.982 and then what you're going to

NOTE Confidence: 0.91749533

00:28:50.982 --> 00:28:52.896 see is that that cell over time,

NOTE Confidence: 0.91749533

00:28:52.896 --> 00:28:54.384 and this is over the course

NOTE Confidence: 0.91749533

00:28:54.384 --> 00:28:55.520 of about seven days,

NOTE Confidence: 0.91749533

00:28:55.520 --> 00:28:56.720 undergoes state specification.

NOTE Confidence: 0.91749533

00:28:56.720 --> 00:28:57.920 If it's blue,

NOTE Confidence: 0.91749533

00:28:57.920 --> 00:28:59.880 it means that downstream of that cell

NOTE Confidence: 0.91749533

00:28:59.880 --> 00:29:02.079 there are both Meg and Erythroid cells.

NOTE Confidence: 0.91749533

00:29:02.080 --> 00:29:02.788 If it's red,

NOTE Confidence: 0.91749533

00:29:02.788 --> 00:29:03.968 it means everything downstream of

NOTE Confidence: 0.91749533

00:29:03.968 --> 00:29:05.717 that is Erythroid and if it's green,

NOTE Confidence: 0.91749533

00:29:05.720 --> 00:29:07.312 it means everything downstream

NOTE Confidence: 0.91749533

00:29:07.312 --> 00:29:10.080 of that is going to be Meg.

NOTE Confidence: 0.91749533

00:29:10.080 --> 00:29:11.410 And there are a lot of things

NOTE Confidence: 0.91749533

00:29:11.410 --> 00:29:12.440 that you can see here.

NOTE Confidence: 0.91749533

00:29:12.440 --> 00:29:14.048 One of them maybe you saw

NOTE Confidence: 0.91749533

00:29:14.048 --> 00:29:15.120 those streaky green lines,

NOTE Confidence: 0.91749533

00:29:15.120 --> 00:29:17.038 the Meg progenitors move a whole lot

NOTE Confidence: 0.91749533

00:29:17.038 --> 00:29:19.119 more than the erythroid progenitors.

NOTE Confidence: 0.91749533

00:29:19.120 --> 00:29:20.632 We're not sure yet what that means

NOTE Confidence: 0.91749533

00:29:20.632 --> 00:29:21.777 and whether it's relevant for

NOTE Confidence: 0.91749533

00:29:21.777 --> 00:29:23.359 what's going on in the bone marrow.

NOTE Confidence: 0.91749533

00:29:23.360 --> 00:29:24.960 But what we do know is in the bone marrow,

NOTE Confidence: 0.91749533

00:29:24.960 --> 00:29:26.080 people have looked at it,

NOTE Confidence: 0.91749533

00:29:26.080 --> 00:29:28.555 Erythroid maturation tends to hurt

NOTE Confidence: 0.91749533

00:29:28.555 --> 00:29:30.552 occur in bundles, whereas megs,

NOTE Confidence: 0.91749533

00:29:30.552 --> 00:29:32.600 they tend to be all over the place.

NOTE Confidence: 0.91749533

00:29:32.600 --> 00:29:34.168 So we think that this might have

NOTE Confidence: 0.91749533

00:29:34.168 --> 00:29:35.582 something to do with the fact

NOTE Confidence: 0.91749533

00:29:35.582 --> 00:29:36.956 that the Meg destined cell is

NOTE Confidence: 0.91749533

00:29:36.956 --> 00:29:38.253 still quite motile and there are

NOTE Confidence: 0.91749533

00:29:38.253 --> 00:29:39.159 other things that you can see.

NOTE Confidence: 0.91749533

00:29:39.160 --> 00:29:42.324 I'll just let's go take show you

NOTE Confidence: 0.91749533

00:29:42.324 --> 00:29:44.127 quickly where you can see that there

NOTE Confidence: 0.91749533

00:29:44.127 --> 00:29:46.213 are blue cells that are still present

NOTE Confidence: 0.91749533

00:29:46.213 --> 00:29:47.838 after multiple rounds of division,

NOTE Confidence: 0.91749533

00:29:47.840 --> 00:29:49.760 but fewer and fewer of them.

NOTE Confidence: 0.91749533

00:29:49.760 --> 00:29:51.720 Some of the blue cells are still

NOTE Confidence: 0.91749533

00:29:51.720 --> 00:29:53.402 here even pretty late when

NOTE Confidence: 0.91749533

00:29:53.402 --> 00:29:55.347 the other ones still haven't

NOTE Confidence: 0.91749533

00:29:55.347 --> 00:29:56.514 undergone fate specification.

NOTE Confidence: 0.91749533

00:29:56.520 --> 00:29:57.688 When we analyze these,

NOTE Confidence: 0.91749533

00:29:57.688 --> 00:29:59.920 one of the first things we saw is.

NOTE Confidence: 0.91749533

00:29:59.920 --> 00:30:01.384 So this is now a tree where the

NOTE Confidence: 0.91749533

00:30:01.384 --> 00:30:02.360 blue cells are bipotent,

NOTE Confidence: 0.91749533

00:30:02.360 --> 00:30:04.106 the red cells are erythroid committed

NOTE Confidence: 0.91749533

00:30:04.106 --> 00:30:06.239 and the green cells are Meg committed.

NOTE Confidence: 0.91749533

00:30:06.240 --> 00:30:07.108 When I say committed,

NOTE Confidence: 0.91749533

00:30:07.108 --> 00:30:08.193 I should probably say destined.

NOTE Confidence: 0.957484911428572

00:30:08.200 --> 00:30:09.516 We don't really know when they committed.

NOTE Confidence: 0.957484911428572

00:30:09.520 --> 00:30:10.840 We just know what the

NOTE Confidence: 0.957484911428572

00:30:10.840 --> 00:30:12.160 cells became at the end.

NOTE Confidence: 0.957484911428572

00:30:12.160 --> 00:30:13.456 One thing you can see though

NOTE Confidence: 0.957484911428572

00:30:13.456 --> 00:30:14.760 is that MEP self renewal,

NOTE Confidence: 0.957484911428572

00:30:14.760 --> 00:30:16.968 this is not something anybody had

NOTE Confidence: 0.957484911428572

00:30:16.968 --> 00:30:19.100 ever known before and it was kind

NOTE Confidence: 0.957484911428572

00:30:19.100 --> 00:30:20.420 of questionable when you look at

NOTE Confidence: 0.957484911428572

00:30:20.420 --> 00:30:21.877 the single cell RNA seek data.

NOTE Confidence: 0.957484911428572

00:30:21.880 --> 00:30:23.875 If you remember we had this graduation,

NOTE Confidence: 0.957484911428572

00:30:23.880 --> 00:30:26.120 I didn't know how long that graduation took.

NOTE Confidence: 0.957484911428572

00:30:26.120 --> 00:30:27.856 Maybe cells just become an MEP and then

NOTE Confidence: 0.957484911428572

00:30:27.856 --> 00:30:29.558 the next day they're mega erythroid.

NOTE Confidence: 0.957484911428572

00:30:29.560 --> 00:30:31.429 But here you can see that the

NOTE Confidence: 0.957484911428572

00:30:31.429 --> 00:30:32.927 bipotent cells can self renew

NOTE Confidence: 0.957484911428572

00:30:32.927 --> 00:30:34.517 and make more bipotent cells.

NOTE Confidence: 0.957484911428572

00:30:34.520 --> 00:30:36.140 Sometimes where one bipotent cell

NOTE Confidence: 0.957484911428572

00:30:36.140 --> 00:30:38.420 makes 2 bipotent cells and times where

NOTE Confidence: 0.957484911428572

00:30:38.420 --> 00:30:40.534 sometimes where it makes 1 bipotent cell

NOTE Confidence: 0.957484911428572

00:30:40.534 --> 00:30:42.679 and one fate Destin cell unique fate.

NOTE Confidence: 0.957484911428572

00:30:42.680 --> 00:30:45.460 And when we and this is just looking

NOTE Confidence: 0.957484911428572

00:30:45.460 --> 00:30:47.113 at the the sometimes when we played

NOTE Confidence: 0.957484911428572

00:30:47.113 --> 00:30:49.040 at MEP we got MK only colonies,

NOTE Confidence: 0.957484911428572

00:30:49.040 --> 00:30:50.642 sometimes when we got we played

NOTE Confidence: 0.957484911428572

00:30:50.642 --> 00:30:52.718 at MEP we got E only colonies.

NOTE Confidence: 0.957484911428572

00:30:52.720 --> 00:30:53.875 So this was another opportunity

NOTE Confidence: 0.957484911428572

00:30:53.875 --> 00:30:55.129 for us to say, well,

NOTE Confidence: 0.957484911428572

00:30:55.129 --> 00:30:57.432 is this different from when we plate

NOTE Confidence: 0.957484911428572

00:30:57.432 --> 00:30:59.371 an erythroid progenitor that we already

NOTE Confidence: 0.957484911428572

00:30:59.371 --> 00:31:02.160 know is E committed or a Meg progenitor?

NOTE Confidence: 0.957484911428572

00:31:02.160 --> 00:31:04.680 And the answer is yes.

NOTE Confidence: 0.957484911428572

00:31:04.680 --> 00:31:06.731 This is a sample tree from an

NOTE Confidence: 0.957484911428572

00:31:06.731 --> 00:31:09.014 MEP that's going to undergo fate

NOTE Confidence: 0.957484911428572

00:31:09.014 --> 00:31:10.798 specification down both lineages.

NOTE Confidence: 0.957484911428572

00:31:10.800 --> 00:31:12.888 Here's one where it's going to

NOTE Confidence: 0.957484911428572

00:31:12.888 --> 00:31:15.399 undergo Meg only or Erythroid only.

NOTE Confidence: 0.957484911428572

00:31:15.400 --> 00:31:17.776 If you compare that when we

NOTE Confidence: 0.957484911428572

00:31:17.776 --> 00:31:19.360 plate the Meg progenitors,

NOTE Confidence: 0.957484911428572

00:31:19.360 --> 00:31:20.600 there aren't very many divisions.

NOTE Confidence: 0.957484911428572

00:31:20.600 --> 00:31:22.846 They make teeny tiny colonies and

NOTE Confidence: 0.957484911428572

00:31:22.846 --> 00:31:24.476 when we play erythroid progenitors,

NOTE Confidence: 0.957484911428572

00:31:24.480 --> 00:31:27.098 what we see is that they reach

NOTE Confidence: 0.957484911428572

00:31:27.098 --> 00:31:28.760 this faster proliferation sooner.

NOTE Confidence: 0.957484911428572

00:31:28.760 --> 00:31:31.168 So they really are downstream of this

NOTE Confidence: 0.957484911428572

00:31:31.168 --> 00:31:33.623 cell that we're seeing here that is

NOTE Confidence: 0.957484911428572

00:31:33.623 --> 00:31:35.602 making a much larger colony with,

NOTE Confidence: 0.957484911428572

00:31:35.602 --> 00:31:37.294 and it doesn't speed up its

NOTE Confidence: 0.957484911428572

00:31:37.294 --> 00:31:38.680 cell division quite so early.

NOTE Confidence: 0.935882898148148

00:31:40.920 --> 00:31:42.656 This is another way of looking at

NOTE Confidence: 0.935882898148148

00:31:42.656 --> 00:31:44.759 the data where what you can see is we

NOTE Confidence: 0.935882898148148

00:31:44.759 --> 00:31:46.503 were able to follow these cells for

NOTE Confidence: 0.935882898148148

00:31:46.503 --> 00:31:48.800 up to 13 generations, a single cell,

NOTE Confidence: 0.935882898148148

00:31:48.800 --> 00:31:51.200 what happens over 13 generations in

NOTE Confidence: 0.935882898148148

00:31:51.200 --> 00:31:53.399 vitro and what you can see is expansion.

NOTE Confidence: 0.935882898148148

00:31:53.400 --> 00:31:55.596 When one MEP makes 2 ME PS tends to

NOTE Confidence: 0.935882898148148

00:31:55.596 --> 00:31:57.995 occur but one is that's where we started.

NOTE Confidence: 0.935882898148148

00:31:58.000 --> 00:31:59.590 We only looked at colonies that

NOTE Confidence: 0.935882898148148

00:31:59.590 --> 00:32:01.239 were going to make both here.

NOTE Confidence: 0.935882898148148

00:32:01.240 --> 00:32:03.408 But what you can see is that you

NOTE Confidence: 0.935882898148148

00:32:03.408 --> 00:32:05.430 really get MEP self renewal where

NOTE Confidence: 0.935882898148148

00:32:05.430 --> 00:32:07.863 you're going to get two expansion from

NOTE Confidence: 0.935882898148148

00:32:07.863 --> 00:32:10.600 MET one MEP to two MEP for the 1st 3

NOTE Confidence: 0.935882898148148

00:32:10.600 --> 00:32:13.358 divisions and then that gradually goes

NOTE Confidence: 0.935882898148148

00:32:13.358 --> 00:32:15.731 away and by the 6th division you're

NOTE Confidence: 0.935882898148148

00:32:15.731 --> 00:32:18.077 not getting one MEP making two MEP.

NOTE Confidence: 0.935882898148148

00:32:18.080 --> 00:32:19.725 In contrast this maintenance division

NOTE Confidence: 0.935882898148148

00:32:19.725 --> 00:32:21.999 where one daughter cells going to be a Meg,

NOTE Confidence: 0.935882898148148

00:32:22.000 --> 00:32:24.016 an MEP and one is going to be fate

NOTE Confidence: 0.935882898148148

00:32:24.016 --> 00:32:25.852 destined that starts to occur at

NOTE Confidence: 0.935882898148148

00:32:25.852 --> 00:32:27.412 approximately the 4th generation and

NOTE Confidence: 0.935882898148148

00:32:27.469 --> 00:32:29.240 that's what we have until the end.

NOTE Confidence: 0.935882898148148

00:32:29.240 --> 00:32:30.598 And with each time you have one

NOTE Confidence: 0.935882898148148

00:32:30.598 --> 00:32:31.640 of these yellow divisions,

NOTE Confidence: 0.935882898148148

00:32:31.640 --> 00:32:34.020 that's when one MEP makes 1 Erythroid

NOTE Confidence: 0.935882898148148

00:32:34.020 --> 00:32:36.439 fate committed and one MK fate committed.

NOTE Confidence: 0.935882898148148

00:32:36.440 --> 00:32:37.960 That's going to be the end of the

NOTE Confidence: 0.935882898148148

00:32:37.960 --> 00:32:39.282 line because we're not going to

NOTE Confidence: 0.935882898148148

00:32:39.282 --> 00:32:39.954 keep following MEP.

NOTE Confidence: 0.935882898148148

00:32:39.960 --> 00:32:42.000 So it really gives us a nice way of looking

NOTE Confidence: 0.935882898148148

00:32:42.050 --> 00:32:43.758 at the changes that occur over time,

NOTE Confidence: 0.935882898148148

00:32:43.760 --> 00:32:46.232 which ends up being highly relevant

NOTE Confidence: 0.935882898148148

00:32:46.232 --> 00:32:47.880 for our predictive models.

NOTE Confidence: 0.935882898148148

00:32:47.880 --> 00:32:49.878 What we wanted to do is come up with

NOTE Confidence: 0.935882898148148

00:32:49.878 --> 00:32:52.100 a mathematical model that gave us the

NOTE Confidence: 0.935882898148148

00:32:52.100 --> 00:32:54.741 outcome that we saw so that we could

NOTE Confidence: 0.935882898148148

00:32:54.741 --> 00:32:56.673 understand the probability that a cell

NOTE Confidence: 0.935882898148148

00:32:56.680 --> 00:32:59.638 would undergo a specific fate decision.

NOTE Confidence: 0.935882898148148

00:32:59.640 --> 00:33:01.472 And this is work done by Everett Thompson

NOTE Confidence: 0.935882898148148

00:33:01.472 --> 00:33:03.838 in my lab who's an amazing graduate student.

NOTE Confidence: 0.935882898148148

00:33:03.840 --> 00:33:06.837 And what he realized is if he used a

NOTE Confidence: 0.935882898148148

00:33:06.837 --> 00:33:09.115 Markov model of these cells that are

NOTE Confidence: 0.935882898148148

00:33:09.115 --> 00:33:11.314 MEP that are expanding to make two

NOTE Confidence: 0.935882898148148

00:33:11.314 --> 00:33:13.300 MEP exhaustion where the MEP makes

NOTE Confidence: 0.935882898148148

00:33:13.300 --> 00:33:15.950 1 erythroid and 1 Meg fate specified

NOTE Confidence: 0.935882898148148

00:33:15.950 --> 00:33:17.875 versus these two maintenance divisions.

NOTE Confidence: 0.935882898148148

00:33:17.880 --> 00:33:20.240 He could model the data that we got

NOTE Confidence: 0.935882898148148

00:33:20.240 --> 00:33:22.862 as long as he had that model change

NOTE Confidence: 0.935882898148148

00:33:22.862 --> 00:33:23.514 over time,

NOTE Confidence: 0.935882898148148

00:33:23.520 --> 00:33:24.794 which is consistent with what I just

NOTE Confidence: 0.935882898148148

00:33:24.794 --> 00:33:25.158 showed you.

NOTE Confidence: 0.935882898148148

00:33:25.160 --> 00:33:27.547 It does change over time the the

NOTE Confidence: 0.935882898148148

00:33:27.547 --> 00:33:29.640 probability that the MEP will self

NOTE Confidence: 0.935882898148148

00:33:29.640 --> 00:33:30.639 renew and expand.

NOTE Confidence: 0.935882898148148

00:33:30.640 --> 00:33:32.593 So when he did that he got the data

NOTE Confidence: 0.935882898148148

00:33:32.593 --> 00:33:33.798 that are plotted here.

NOTE Confidence: 0.935882898148148

00:33:33.800 --> 00:33:36.579 So what you're seeing here is the

NOTE Confidence: 0.935882898148148

00:33:36.579 --> 00:33:38.798 the broadbands shown here in blue,

NOTE Confidence: 0.935882898148148

00:33:38.800 --> 00:33:40.644 purple, Aqua and yellow.

NOTE Confidence: 0.935882898148148

00:33:40.644 --> 00:33:44.359 That is the data predicted by the model.

NOTE Confidence: 0.935882898148148

00:33:44.360 --> 00:33:47.920 And then in the dotted line is the.

NOTE Confidence: 0.935882898148148

00:33:47.920 --> 00:33:50.080 I want to make sure I say the right thing.

NOTE Confidence: 0.935882898148148

00:33:50.080 --> 00:33:50.346 Yeah.

NOTE Confidence: 0.935882898148148

00:33:50.346 --> 00:33:52.474 And the dotted line is the in blue

NOTE Confidence: 0.935882898148148

00:33:52.474 --> 00:33:53.918 is the observed data.

NOTE Confidence: 0.935882898148148

00:33:53.920 --> 00:33:55.976 So what you can see is we really

NOTE Confidence: 0.935882898148148

00:33:55.976 --> 00:33:58.265 are very closely modeling what the

NOTE Confidence: 0.935882898148148

00:33:58.265 --> 00:34:01.200 actual data are for the exhaustion,

NOTE Confidence: 0.935882898148148

00:34:01.200 --> 00:34:02.568 expansion, maintenance and maintenance.

NOTE Confidence: 0.935882898148148

00:34:02.568 --> 00:34:04.840 The way to look at this is,

NOTE Confidence: 0.935882898148148

00:34:04.840 --> 00:34:05.470 for example,

NOTE Confidence: 0.935882898148148

00:34:05.470 --> 00:34:07.675 if you just look at Generation 4,

NOTE Confidence: 0.935882898148148

00:34:07.680 --> 00:34:09.040 if you have an MEP,

NOTE Confidence: 0.935882898148148

00:34:09.040 --> 00:34:11.770 their chances are 46% chance of

NOTE Confidence: 0.935882898148148

00:34:11.770 --> 00:34:14.822 expansion or one MEP makes 2 MEP,

NOTE Confidence: 0.935882898148148

00:34:14.822 --> 00:34:16.226 28% chance that you're

NOTE Confidence: 0.935882898148148

00:34:16.226 --> 00:34:17.630 going to get maintenance

NOTE Confidence: 0.891759870909091

00:34:17.700 --> 00:34:19.688 plus E, 9% chance of maintenance plus

NOTE Confidence: 0.891759870909091

00:34:19.688 --> 00:34:22.000 MK and a 17% chance of exhaustion.

NOTE Confidence: 0.891759870909091

00:34:22.000 --> 00:34:23.910 Well, that kind of models our

NOTE Confidence: 0.891759870909091

00:34:23.910 --> 00:34:26.300 outcome in our CFU where we get about

NOTE Confidence: 0.891759870909091

00:34:26.300 --> 00:34:28.336 50% of the colonies have Mega and

NOTE Confidence: 0.891759870909091

00:34:28.336 --> 00:34:30.231 Erythroid and the other ones are

NOTE Confidence: 0.891759870909091

00:34:30.231 --> 00:34:32.199 Unilineage MK only and Erythroid only.

NOTE Confidence: 0.891759870909091

00:34:32.200 --> 00:34:35.288 And then similarly you can look at another

NOTE Confidence: 0.891759870909091

00:34:35.288 --> 00:34:37.040 generation and get additional data.

NOTE Confidence: 0.915327742173913

00:34:39.120 --> 00:34:41.010 She Vanessa got a huge amount of

NOTE Confidence: 0.915327742173913

00:34:41.010 --> 00:34:43.424 data out of this and I just want to

NOTE Confidence: 0.915327742173913

00:34:43.424 --> 00:34:45.320 show you one other part of that.

NOTE Confidence: 0.915327742173913

00:34:45.320 --> 00:34:47.824 And what she did is she analyzed the

NOTE Confidence: 0.915327742173913

00:34:47.824 --> 00:34:50.402 length of the cell cycle and whether

NOTE Confidence: 0.915327742173913

00:34:50.402 --> 00:34:52.669 that predicted output and it wasn't

NOTE Confidence: 0.915327742173913

00:34:52.669 --> 00:34:55.680 as simple as we had hoped, but we did

NOTE Confidence: 0.915327742173913

00:34:55.680 --> 00:34:57.480 get some statistically significant data.

NOTE Confidence: 0.915327742173913

00:34:57.480 --> 00:35:00.360 The data that we got is that MEP

NOTE Confidence: 0.915327742173913

00:35:00.360 --> 00:35:03.576 that are cycling slower are going

NOTE Confidence: 0.915327742173913

00:35:03.576 --> 00:35:06.516 to be the MK destined.

NOTE Confidence: 0.915327742173913

00:35:06.520 --> 00:35:07.660 Remember MEP cycling?

NOTE Confidence: 0.915327742173913

00:35:07.660 --> 00:35:09.560 I have to remember exactly.

NOTE Confidence: 0.915327742173913

00:35:09.560 --> 00:35:10.840 So there was no difference.

NOTE Confidence: 0.915327742173913

00:35:10.840 --> 00:35:12.800 And this is where this was disappointing.

NOTE Confidence: 0.915327742173913

00:35:12.800 --> 00:35:15.760 There was no difference in the cell cycle

NOTE Confidence: 0.915327742173913

00:35:15.760 --> 00:35:19.918 interval between MEP and E destined cells.

NOTE Confidence: 0.915327742173913

00:35:19.920 --> 00:35:22.260 I thought that we would have seen that the E

NOTE Confidence: 0.915327742173913

00:35:22.320 --> 00:35:24.131 destined cells had a faster proliferation,

NOTE Confidence: 0.915327742173913

00:35:24.131 --> 00:35:26.357 but that's not what we saw.

NOTE Confidence: 0.915327742173913

00:35:26.360 --> 00:35:27.122 But we did.

NOTE Confidence: 0.915327742173913

00:35:27.122 --> 00:35:29.866 What we did see is that once we and with

NOTE Confidence: 0.915327742173913

00:35:29.866 --> 00:35:31.959 MK destined it was a little slower.

NOTE Confidence: 0.915327742173913

00:35:31.960 --> 00:35:34.039 That's the point I wanted to make.

NOTE Confidence: 0.915327742173913

00:35:34.040 --> 00:35:35.360 So there was a slowing,

NOTE Confidence: 0.915327742173913

00:35:35.360 --> 00:35:37.068 as if the cell was dividing more

NOTE Confidence: 0.915327742173913

00:35:37.068 --> 00:35:38.691 slowly there was a very good chance

NOTE Confidence: 0.915327742173913

00:35:38.691 --> 00:35:40.599 that it was going to be MK destined.

NOTE Confidence: 0.915327742173913

00:35:40.600 --> 00:35:42.553 And then if you looked at the MKP themselves,

NOTE Confidence: 0.915327742173913

00:35:42.560 --> 00:35:42.920 they are,

NOTE Confidence: 0.915327742173913

00:35:42.920 --> 00:35:44.360 they're known to have a slower cell cycle.

NOTE Confidence: 0.915327742173913

00:35:44.360 --> 00:35:45.360 I already told you that.

NOTE Confidence: 0.915327742173913

00:35:45.360 --> 00:35:46.900 But this was really the the new

NOTE Confidence: 0.915327742173913

00:35:46.900 --> 00:35:48.490 data was this MK Destined having

NOTE Confidence: 0.915327742173913

00:35:48.490 --> 00:35:49.955 a slightly slower cell cycle.

NOTE Confidence: 0.915327742173913

00:35:49.960 --> 00:35:51.640 So not quite as clear as we would have liked,

NOTE Confidence: 0.915327742173913

00:35:51.640 --> 00:35:53.440 but that's what the data show

NOTE Confidence: 0.828415577142857

00:35:56.280 --> 00:35:57.918 this. So just this is this time

NOTE Confidence: 0.828415577142857

00:35:57.918 --> 00:35:59.864 lapse imaging is now a tool in the

NOTE Confidence: 0.828415577142857

00:35:59.864 --> 00:36:01.400 laboratory that we are enjoying using.

NOTE Confidence: 0.828415577142857

00:36:01.400 --> 00:36:03.062 If anybody wants to collaborate and

NOTE Confidence: 0.828415577142857

00:36:03.062 --> 00:36:04.839 use this tool just let us know.

NOTE Confidence: 0.828415577142857

00:36:04.840 --> 00:36:07.738 It's one of the tools that's offered by the

NOTE Confidence: 0.828415577142857

00:36:07.738 --> 00:36:11.720 Yale Center of Excellence in Hematology.

NOTE Confidence: 0.828415577142857

00:36:11.720 --> 00:36:13.680 So last story, plenty of time I

NOTE Confidence: 0.828415577142857

00:36:13.680 --> 00:36:15.955 wanted to tell you about expansion

NOTE Confidence: 0.828415577142857

00:36:15.955 --> 00:36:18.240 microscopy to probe hematopoietic cells.

NOTE Confidence: 0.828415577142857

00:36:18.240 --> 00:36:20.120 So what is expansion microscopy?

NOTE Confidence: 0.828415577142857

00:36:20.120 --> 00:36:23.032 This is a way of doing super

NOTE Confidence: 0.828415577142857

00:36:23.032 --> 00:36:25.398 resolution microscopy using a confocal

NOTE Confidence: 0.828415577142857

00:36:25.398 --> 00:36:26.992 microscope and that really opens

NOTE Confidence: 0.828415577142857

00:36:26.992 --> 00:36:29.722 up the door to all of those of us

NOTE Confidence: 0.828415577142857

00:36:29.722 --> 00:36:31.477 who don't do electron microscopy.

NOTE Confidence: 0.828415577142857

00:36:31.480 --> 00:36:33.480 And even if you do do electron microscopy,

NOTE Confidence: 0.828415577142857

00:36:33.480 --> 00:36:35.560 you know it's very difficult to do any

NOTE Confidence: 0.828415577142857

00:36:35.560 --> 00:36:37.505 kind of immuno analysis because you're

NOTE Confidence: 0.828415577142857

00:36:37.505 --> 00:36:40.235 really limited to the size of the gold

NOTE Confidence: 0.828415577142857

00:36:40.235 --> 00:36:42.440 balls that are attached to your antibody.

NOTE Confidence: 0.828415577142857

00:36:42.440 --> 00:36:44.430 So you maybe can look at two things at the

NOTE Confidence: 0.828415577142857

00:36:44.480 --> 00:36:46.514 same time and maybe can see where they are.

NOTE Confidence: 0.828415577142857

00:36:46.520 --> 00:36:48.936 Here you have a confocal you can do

NOTE Confidence: 0.828415577142857

00:36:48.936 --> 00:36:50.518 immunofluorescence from for some antigens,

NOTE Confidence: 0.828415577142857

00:36:50.520 --> 00:36:52.319 not for every antigen with the expansion.

NOTE Confidence: 0.956286988888889

00:36:55.360 --> 00:36:57.475 So this is just to get you guys interested,

NOTE Confidence: 0.956286988888889

00:36:57.480 --> 00:36:58.960 if you're not a pathologist

NOTE Confidence: 0.956286988888889

00:36:58.960 --> 00:37:00.440 in looking at mega karyocytes,

NOTE Confidence: 0.956286988888889

00:37:00.440 --> 00:37:02.198 they happen to be the most beautiful

NOTE Confidence: 0.956286988888889

00:37:02.200 --> 00:37:03.999 cell in the body according to me.

NOTE Confidence: 0.956286988888889

00:37:04.000 --> 00:37:05.760 And what you can see is they're very,

NOTE Confidence: 0.956286988888889

00:37:05.760 --> 00:37:08.560 very large, hence the name mega karyocyte.

NOTE Confidence: 0.956286988888889

00:37:08.560 --> 00:37:10.160 What we're looking at here

NOTE Confidence: 0.956286988888889

00:37:10.160 --> 00:37:12.220 is a bunch of blood cells.

NOTE Confidence: 0.956286988888889

00:37:12.220 --> 00:37:13.995 These are your normal neutrophils.

NOTE Confidence: 0.956286988888889

00:37:14.000 --> 00:37:15.400 You can see the size of their nucleus,

NOTE Confidence: 0.956286988888889

00:37:15.400 --> 00:37:17.140 it's about 8 microns and

NOTE Confidence: 0.956286988888889

00:37:17.140 --> 00:37:18.880 this is a mega karyocyte.

NOTE Confidence: 0.956286988888889

00:37:18.880 --> 00:37:20.656 It's a single cell.

NOTE Confidence: 0.956286988888889

00:37:20.656 --> 00:37:23.817 It's got this gigantic nucleus and a

NOTE Confidence: 0.956286988888889

00:37:23.817 --> 00:37:26.840 gigantic cell and what this nucleus is,

NOTE Confidence: 0.956286988888889

00:37:26.840 --> 00:37:27.719 is it's polyploid.

NOTE Confidence: 0.956286988888889

00:37:27.719 --> 00:37:29.477 It's got the cell has divide,

NOTE Confidence: 0.956286988888889

00:37:29.480 --> 00:37:31.258 the DNA has divided and the cell

NOTE Confidence: 0.956286988888889

00:37:31.258 --> 00:37:32.639 has gotten bigger and bigger,

NOTE Confidence: 0.956286988888889

00:37:32.640 --> 00:37:34.080 but the cell has not divided.

NOTE Confidence: 0.956286988888889

00:37:34.080 --> 00:37:35.048 So you have many.

NOTE Confidence: 0.956286988888889

00:37:35.048 --> 00:37:37.360 You can get four and eight and 1632,

NOTE Confidence: 0.956286988888889

00:37:37.360 --> 00:37:40.560 whatever, up to 128 clearly.

NOTE Confidence: 0.956286988888889

00:37:40.560 --> 00:37:42.716 And then this part of this cell,

NOTE Confidence: 0.956286988888889

00:37:42.720 --> 00:37:43.780 which is super interesting

NOTE Confidence: 0.956286988888889

00:37:43.780 --> 00:37:44.840 and hard to describe,

NOTE Confidence: 0.956286988888889

00:37:44.840 --> 00:37:46.640 but you're about to see what it is.

NOTE Confidence: 0.956286988888889

00:37:46.640 --> 00:37:50.426 It's not a single cell membrane

NOTE Confidence: 0.956286988888889

00:37:50.426 --> 00:37:52.319 surrounding a cytopus.

NOTE Confidence: 0.956286988888889

00:37:52.320 --> 00:37:53.361 Well, it is,

NOTE Confidence: 0.956286988888889

00:37:53.361 --> 00:37:55.443 but the cell membrane is invaginated

NOTE Confidence: 0.956286988888889

00:37:55.443 --> 00:37:57.558 all throughout that cytoplasm.

NOTE Confidence: 0.956286988888889

00:37:57.560 --> 00:38:00.320 And way you can see that is from this movie.

NOTE Confidence: 0.956286988888889

00:38:00.320 --> 00:38:01.120 So this is a movie.

NOTE Confidence: 0.956286988888889

00:38:01.120 --> 00:38:03.720 It was published in 1999 by Joe Italiano,

NOTE Confidence: 0.956286988888889

00:38:03.720 --> 00:38:05.405 who's an amazing mega karyocyte

NOTE Confidence: 0.956286988888889

00:38:05.405 --> 00:38:06.753 scientist up at Harvard.

NOTE Confidence: 0.956286988888889

00:38:06.760 --> 00:38:08.596 This is a single mega karyocyte.

NOTE Confidence: 0.956286988888889

00:38:08.600 --> 00:38:09.611 Here's its nucleus.

NOTE Confidence: 0.956286988888889

00:38:09.611 --> 00:38:11.633 It's starting to make pro platelets.

NOTE Confidence: 0.956286988888889

00:38:11.640 --> 00:38:13.278 And the thing that's amazing about

NOTE Confidence: 0.956286988888889

00:38:13.278 --> 00:38:15.306 this movie is you can see that

NOTE Confidence: 0.956286988888889

00:38:15.306 --> 00:38:16.776 the cytoplasm is basically going

NOTE Confidence: 0.956286988888889

00:38:16.776 --> 00:38:18.722 to unravel to release the pro

NOTE Confidence: 0.956286988888889

00:38:18.722 --> 00:38:20.352 platelets that then become platelets.

NOTE Confidence: 0.908491521428571

00:38:25.560 --> 00:38:27.996 So all that membrane system was inside,

NOTE Confidence: 0.908491521428571

00:38:28.000 --> 00:38:30.288 it was all packaged and then it just

NOTE Confidence: 0.908491521428571

00:38:30.288 --> 00:38:33.030 had to be induced to to unravel itself

NOTE Confidence: 0.908491521428571

00:38:33.030 --> 00:38:35.080 and release these pro platelets.

NOTE Confidence: 0.908491521428571

00:38:35.080 --> 00:38:37.796 So yeah, it's a very cool movie.

NOTE Confidence: 0.908491521428571

00:38:37.800 --> 00:38:40.710 When people then look at megacary sites, they

NOTE Confidence: 0.908491521428571

00:38:40.710 --> 00:38:43.160 want to see that demarcation membrane system,

NOTE Confidence: 0.908491521428571

00:38:43.160 --> 00:38:46.040 that invagination of the plasma membrane.

NOTE Confidence: 0.908491521428571

00:38:46.040 --> 00:38:47.640 And we're doing this using

NOTE Confidence: 0.908491521428571

00:38:47.640 --> 00:38:48.280 expansion microscopy.

NOTE Confidence: 0.908491521428571

00:38:48.280 --> 00:38:49.918 So what is it, expansion microscopy?

NOTE Confidence: 0.908491521428571

00:38:49.920 --> 00:38:53.760 It's been developed in multiple laboratories.

NOTE Confidence: 0.908491521428571

00:38:53.760 --> 00:38:55.056 Neither of these labs,

NOTE Confidence: 0.908491521428571

00:38:55.056 --> 00:38:56.676 York Broersdorf or Yong Shinzhao's,

NOTE Confidence: 0.908491521428571

00:38:56.680 --> 00:38:57.916 was the first to do it.

NOTE Confidence: 0.908491521428571

00:38:57.920 --> 00:38:59.432 But these are the two people

NOTE Confidence: 0.908491521428571

00:38:59.432 --> 00:39:00.440 that we're collaborating with.

NOTE Confidence: 0.908491521428571

00:39:00.440 --> 00:39:01.478 Many of you may know York.

NOTE Confidence: 0.908491521428571

00:39:01.480 --> 00:39:02.444 He's here at Yale.

NOTE Confidence: 0.908491521428571

00:39:02.444 --> 00:39:04.148 He does beautiful work with Pan XM

NOTE Confidence: 0.908491521428571

00:39:04.148 --> 00:39:06.308 that I'll show you the I And Yong

NOTE Confidence: 0.908491521428571

00:39:06.308 --> 00:39:07.993 Shinzhao is at Carnegie Mellon.

NOTE Confidence: 0.908491521428571

00:39:08.000 --> 00:39:12.319 He has a different approach called magnify.

NOTE Confidence: 0.908491521428571

00:39:12.320 --> 00:39:14.732 And what you can see is that you take

NOTE Confidence: 0.908491521428571

00:39:14.732 --> 00:39:17.443 your cell and here we're just looking

NOTE Confidence: 0.908491521428571

00:39:17.443 --> 00:39:19.361 at different the mitochondria and

NOTE Confidence: 0.908491521428571

00:39:19.361 --> 00:39:21.513 the Golgi here in the cell and you

NOTE Confidence: 0.908491521428571

00:39:21.520 --> 00:39:25.078 polymerize polyacrylamide gel into the cell,

NOTE Confidence: 0.908491521428571

00:39:25.080 --> 00:39:27.200 hit it and it cross links with it.

NOTE Confidence: 0.908491521428571

00:39:27.200 --> 00:39:29.534 You then expand that because there's

NOTE Confidence: 0.908491521428571

00:39:29.534 --> 00:39:32.199 acrylamide in there and sodium acrylate.

NOTE Confidence: 0.908491521428571

00:39:32.200 --> 00:39:34.797 Sodium acrylate is what's in babies diapers.

NOTE Confidence: 0.908491521428571

00:39:34.800 --> 00:39:35.514 It's very,

NOTE Confidence: 0.908491521428571

00:39:35.514 --> 00:39:36.228 very absorptive.

NOTE Confidence: 0.908491521428571

00:39:36.228 --> 00:39:39.081 So if you have sodium acrylate and then

NOTE Confidence: 0.908491521428571

00:39:39.081 --> 00:39:41.001 you add water everything expands so

NOTE Confidence: 0.908491521428571

00:39:41.001 --> 00:39:43.311 you get this huge expansion then what

NOTE Confidence: 0.908491521428571

00:39:43.311 --> 00:39:46.618 they do in the boomers Dorf's lab is

NOTE Confidence: 0.908491521428571

00:39:46.618 --> 00:39:49.900 they stop that get rid of the cross

NOTE Confidence: 0.908491521428571

00:39:49.900 --> 00:39:52.160 linking re embedded and do it again.

NOTE Confidence: 0.908491521428571

00:39:52.160 --> 00:39:54.374 So they can get up to 16 to 20

NOTE Confidence: 0.908491521428571

00:39:54.374 --> 00:39:56.597 fold expansion of a single cell.

NOTE Confidence: 0.908491521428571

00:39:56.600 --> 00:39:58.608 With Magnify you get about a 10 fold

NOTE Confidence: 0.908491521428571

00:39:58.608 --> 00:40:00.373 expansion and I'll tell you about the

NOTE Confidence: 0.908491521428571

00:40:00.373 --> 00:40:02.438 differences but we we do both in the lab.

NOTE Confidence: 0.908491521428571

00:40:02.440 --> 00:40:04.752 I mean the idea is you type take

NOTE Confidence: 0.908491521428571

00:40:04.752 --> 00:40:06.838 one thing that was really little

NOTE Confidence: 0.908491521428571

00:40:06.838 --> 00:40:08.633 and now it's really big.

NOTE Confidence: 0.908491521428571

00:40:08.640 --> 00:40:12.167 This is data from your Goersdorf's lab using

NOTE Confidence: 0.908491521428571

00:40:12.167 --> 00:40:15.600 the Pan XM his two fold expansion approach.

NOTE Confidence: 0.908491521428571

00:40:15.600 --> 00:40:18.240 What you can see in these cells is

NOTE Confidence: 0.908491521428571

00:40:18.240 --> 00:40:21.160 an NHS Ester just stains proteins.

NOTE Confidence: 0.908491521428571

00:40:21.160 --> 00:40:23.225 So it gives you something that's very

NOTE Confidence: 0.908491521428571

00:40:23.225 --> 00:40:25.276 similar to what you might see on EM.

NOTE Confidence: 0.908491521428571

00:40:25.280 --> 00:40:26.880 And you see this beautiful

NOTE Confidence: 0.908491521428571

00:40:26.880 --> 00:40:28.480 Golgi apparatus in a cell.

NOTE Confidence: 0.908491521428571

00:40:28.480 --> 00:40:31.198 This is just he LA cells.

NOTE Confidence: 0.908491521428571

00:40:31.200 --> 00:40:33.642 They can actually get antibodies to

NOTE Confidence: 0.908491521428571

00:40:33.642 --> 00:40:36.433 work that allow them to localize whether

NOTE Confidence: 0.908491521428571

00:40:36.433 --> 00:40:39.378 a protein is on the outside or the

NOTE Confidence: 0.908491521428571

00:40:39.378 --> 00:40:41.248 inside of this of the mitochondria.

NOTE Confidence: 0.908491521428571

00:40:41.248 --> 00:40:43.670 And So what you can see here is

NOTE Confidence: 0.908491521428571

00:40:43.670 --> 00:40:45.679 when they stain with anti Cox four,

NOTE Confidence: 0.908491521428571

00:40:45.680 --> 00:40:48.277 it's on the inside of the mitochondria.

NOTE Confidence: 0.908491521428571

00:40:48.280 --> 00:40:49.624 When they stain with anti Tom 20

NOTE Confidence: 0.908491521428571

00:40:49.624 --> 00:40:51.288 which is known to be on the outside

NOTE Confidence: 0.908491521428571

00:40:51.288 --> 00:40:51.915 of the mitochondria,

NOTE Confidence: 0.908491521428571

00:40:51.920 --> 00:40:53.045 you can see this different

NOTE Confidence: 0.908491521428571

00:40:53.045 --> 00:40:54.106 pattern and it's really,

NOTE Confidence: 0.908491521428571

00:40:54.106 --> 00:40:55.542 really beautiful how you

NOTE Confidence: 0.908491521428571

00:40:55.542 --> 00:40:57.197 can clearly see the Cox,

NOTE Confidence: 0.908491521428571

00:40:57.200 --> 00:40:59.480 the Tom 20 is on the outside and

NOTE Confidence: 0.908491521428571

00:40:59.480 --> 00:41:02.118 the Cox 9 is on the inside Cox four,

NOTE Confidence: 0.908491521428571

00:41:02.120 --> 00:41:02.463 sorry.

NOTE Confidence: 0.908491521428571

00:41:02.463 --> 00:41:04.521 So just beautiful imaging that we

NOTE Confidence: 0.908491521428571

00:41:04.521 --> 00:41:07.324 want to be able to use in now in

NOTE Confidence: 0.908491521428571

00:41:07.324 --> 00:41:09.200 mega carry sites and platelets.

NOTE Confidence: 0.908491521428571

00:41:09.200 --> 00:41:11.769 This is a comparison of Magnify which

NOTE Confidence: 0.908491521428571

00:41:11.769 --> 00:41:14.639 is from Yong Shin Zhao's lab and the

NOTE Confidence: 0.658709767777778

00:41:14.640 --> 00:41:17.079 Pan XM that is in your Boomer source lab.

NOTE Confidence: 0.658709767777778

00:41:17.080 --> 00:41:20.750 And we really takes the best of both in some

NOTE Confidence: 0.658709767777778

00:41:20.835 --> 00:41:22.880 of our assays York Boomersdorf's approach.

NOTE Confidence: 0.658709767777778

00:41:22.880 --> 00:41:25.760 The Pan XM gives you much better resolution.

NOTE Confidence: 0.658709767777778

00:41:25.760 --> 00:41:28.651 No doubt you're getting 16X expansion and

NOTE Confidence: 0.658709767777778

00:41:28.651 --> 00:41:31.079 you're really preserving morphology better.

NOTE Confidence: 0.658709767777778

00:41:31.080 --> 00:41:34.599 However, it takes a lot of time and effort.

NOTE Confidence: 0.658709767777778

00:41:34.600 --> 00:41:36.520 In contrast,

NOTE Confidence: 0.658709767777778

00:41:36.520 --> 00:41:37.576 Yongshin's approach called Magnify,

NOTE Confidence: 0.658709767777778

00:41:37.576 --> 00:41:39.160 just takes one to three days.

NOTE Confidence: 0.658709767777778

00:41:39.160 --> 00:41:41.460 It's less than an hour of hands on time per

NOTE Confidence: 0.658709767777778

00:41:41.520 --> 00:41:43.879 day and there's no special equipment needed.

NOTE Confidence: 0.658709767777778

00:41:43.880 --> 00:41:45.205 You don't need this nitrogen

NOTE Confidence: 0.658709767777778

00:41:45.205 --> 00:41:47.120 tank and you get less expansion,

NOTE Confidence: 0.658709767777778

00:41:47.120 --> 00:41:48.120 but it's still quite beautiful.

NOTE Confidence: 0.658709767777778

00:41:48.120 --> 00:41:49.338 So I'll show you some data

NOTE Confidence: 0.658709767777778

00:41:49.338 --> 00:41:50.440 that we have for each.

NOTE Confidence: 0.658709767777778

00:41:50.440 --> 00:41:53.716 And this is not an expensive thing to do.

NOTE Confidence: 0.658709767777778

00:41:53.720 --> 00:41:56.216 This is just a beautiful image

NOTE Confidence: 0.658709767777778

00:41:56.216 --> 00:41:58.940 that comes from the that we did in

NOTE Confidence: 0.658709767777778

00:41:58.940 --> 00:42:00.000 collaboration with your Brewers.

NOTE Confidence: 0.658709767777778

00:42:00.000 --> 00:42:02.048 Dorf's lab and your runs the imaging core

NOTE Confidence: 0.658709767777778

00:42:02.048 --> 00:42:04.437 for the Center of Excellence in Hematology.

NOTE Confidence: 0.658709767777778

00:42:04.440 --> 00:42:06.800 And what you see here is a pan XM image.

NOTE Confidence: 0.658709767777778

00:42:06.800 --> 00:42:09.638 So that's the 16 fold increase,

NOTE Confidence: 0.658709767777778

00:42:09.640 --> 00:42:10.486 20 fold increase.

NOTE Confidence: 0.658709767777778

00:42:10.486 --> 00:42:13.080 And they pan stained it with the NHS Ester,

NOTE Confidence: 0.658709767777778

00:42:13.080 --> 00:42:16.560 which stains all proteins and with M cling.

NOTE Confidence: 0.658709767777778

00:42:16.560 --> 00:42:18.360 The nice thing about M cling is it bind,

NOTE Confidence: 0.658709767777778

00:42:18.360 --> 00:42:20.160 you stain the cells before you expand them.

NOTE Confidence: 0.658709767777778

00:42:20.160 --> 00:42:21.660 It binds to membranes,

NOTE Confidence: 0.658709767777778

00:42:21.660 --> 00:42:23.160 it binds to lipids.

NOTE Confidence: 0.658709767777778

00:42:23.160 --> 00:42:26.200 And this is allowing us to start to see this

NOTE Confidence: 0.658709767777778

00:42:26.279 --> 00:42:29.119 invaginated membrane throughout the cell.

NOTE Confidence: 0.658709767777778

00:42:29.120 --> 00:42:31.190 And we're getting better and better

NOTE Confidence: 0.658709767777778

00:42:31.190 --> 00:42:32.952 images of this invagination that

NOTE Confidence: 0.658709767777778

00:42:32.952 --> 00:42:34.890 tells that shows us the demarcation

NOTE Confidence: 0.658709767777778

00:42:34.890 --> 00:42:36.960 membrane system of the megakaryocytes.

NOTE Confidence: 0.918802040909091

00:42:39.120 --> 00:42:40.674 So here's another way of looking at

NOTE Confidence: 0.918802040909091

00:42:40.674 --> 00:42:41.640 this demarcation membrane system.

NOTE Confidence: 0.918802040909091

00:42:41.640 --> 00:42:43.809 Now not with the M cling but just with

NOTE Confidence: 0.918802040909091

00:42:43.809 --> 00:42:45.836 the pan stain of all the proteins.

NOTE Confidence: 0.918802040909091

00:42:45.840 --> 00:42:48.367 This is an electron microscopy image and

NOTE Confidence: 0.918802040909091

00:42:48.367 --> 00:42:51.640 this is from our expanded whole bone marrow.

NOTE Confidence: 0.918802040909091

00:42:51.640 --> 00:42:53.600 This is Mina Shu gave us this slide.

NOTE Confidence: 0.918802040909091

00:42:53.600 --> 00:42:55.440 So this is expanded bone

NOTE Confidence: 0.918802040909091

00:42:55.440 --> 00:42:57.280 marrow from human FFPE tissue.

NOTE Confidence: 0.918802040909091

00:42:57.280 --> 00:42:59.701 And what you can see is that this PAN

NOTE Confidence: 0.918802040909091

00:42:59.701 --> 00:43:02.263 XM really shows you the demarcation

NOTE Confidence: 0.918802040909091

00:43:02.263 --> 00:43:04.951 membrane system similarly to what you

NOTE Confidence: 0.918802040909091

00:43:04.951 --> 00:43:07.315 can see with the electron microscopy.

NOTE Confidence: 0.918802040909091

00:43:07.320 --> 00:43:08.740 Here's another expanded thing.

NOTE Confidence: 0.918802040909091

00:43:08.740 --> 00:43:10.515 This is now from magnify,

NOTE Confidence: 0.918802040909091

00:43:10.520 --> 00:43:11.936 showing that we have some antigens

NOTE Confidence: 0.918802040909091

00:43:11.936 --> 00:43:12.880 that we can identify.

NOTE Confidence: 0.918802040909091

00:43:12.880 --> 00:43:15.016 We can identify CD 61 shown

NOTE Confidence: 0.918802040909091

00:43:15.016 --> 00:43:16.440 in green and thrombospondin.

NOTE Confidence: 0.918802040909091

00:43:16.440 --> 00:43:19.240 So these are megacaryocytes and

NOTE Confidence: 0.918802040909091

00:43:19.240 --> 00:43:21.974 these green and red vesicles are

NOTE Confidence: 0.918802040909091

00:43:21.974 --> 00:43:23.456 actually the granules that are going

NOTE Confidence: 0.918802040909091

00:43:23.456 --> 00:43:24.918 to become the platelet granules,

NOTE Confidence: 0.918802040909091

00:43:24.920 --> 00:43:26.320 the alpha granules that have

NOTE Confidence: 0.918802040909091

00:43:26.320 --> 00:43:27.720 within them the thrombus bonded.

NOTE Confidence: 0.959589074

00:43:29.760 --> 00:43:30.640 And this is an image.

NOTE Confidence: 0.959589074

00:43:30.640 --> 00:43:32.476 I just can't get it out of my mind.

NOTE Confidence: 0.959589074

00:43:32.480 --> 00:43:33.680 But we haven't seen this again,

NOTE Confidence: 0.959589074

00:43:33.680 --> 00:43:34.640 we haven't done this.

NOTE Confidence: 0.959589074

00:43:34.640 --> 00:43:36.590 Again, this is again the formal

NOTE Confidence: 0.959589074

00:43:36.590 --> 00:43:38.385 and fixed paraffin embedded tissue

NOTE Confidence: 0.959589074

00:43:38.385 --> 00:43:40.935 from Mina shoe where we just

NOTE Confidence: 0.959589074

00:43:40.935 --> 00:43:44.000 did a pan stain after expansion.

NOTE Confidence: 0.959589074

00:43:44.000 --> 00:43:45.330 And I can't get over this little

NOTE Confidence: 0.959589074

00:43:45.330 --> 00:43:46.560 hole in the megacaryocyte.

NOTE Confidence: 0.959589074

00:43:46.560 --> 00:43:48.639 I really think that this might be

NOTE Confidence: 0.959589074

00:43:48.639 --> 00:43:50.279 where the invagination is happening,

NOTE Confidence: 0.959589074

00:43:50.280 --> 00:43:51.715 but we have to see it more.

NOTE Confidence: 0.959589074

00:43:51.720 --> 00:43:52.770 But I'm showing it to you

NOTE Confidence: 0.959589074

00:43:52.770 --> 00:43:53.920 because this is a pathology,

NOTE Confidence: 0.959589074

00:43:53.920 --> 00:43:55.798 grand rounds and it's so beautiful.

NOTE Confidence: 0.959589074

00:43:55.800 --> 00:43:57.396 These are autofluorescent red blood cells.

NOTE Confidence: 0.959589074

00:43:57.400 --> 00:43:59.824 On the on the outside it's

NOTE Confidence: 0.959589074

00:43:59.824 --> 00:44:01.440 just your gigantic nucleus.

NOTE Confidence: 0.959589074

00:44:01.440 --> 00:44:03.665 What what we've been quite

NOTE Confidence: 0.959589074

00:44:03.665 --> 00:44:05.640 successful at is using this to

NOTE Confidence: 0.959589074

00:44:05.640 --> 00:44:07.260 look at platelets and this is

NOTE Confidence: 0.959589074

00:44:07.322 --> 00:44:09.317 work that was done by Max Carlino.

NOTE Confidence: 0.959589074

00:44:09.320 --> 00:44:10.976 Some of you may know he's a first

NOTE Confidence: 0.959589074

00:44:10.976 --> 00:44:12.359 year graduate student of pathology,

NOTE Confidence: 0.959589074

00:44:12.360 --> 00:44:13.928 but he worked in my lab before

NOTE Confidence: 0.959589074

00:44:13.928 --> 00:44:15.782 that and he worked on this

NOTE Confidence: 0.959589074

00:44:15.782 --> 00:44:17.278 expansion microscopy on platelets.

NOTE Confidence: 0.959589074

00:44:17.280 --> 00:44:19.848 This is just an ultra an electromic

NOTE Confidence: 0.959589074

00:44:19.848 --> 00:44:21.960 graph view of a platelet and you can

NOTE Confidence: 0.959589074

00:44:22.022 --> 00:44:23.702 see that there are dense granules

NOTE Confidence: 0.959589074

00:44:23.702 --> 00:44:25.360 and there are alpha granules.

NOTE Confidence: 0.959589074

00:44:25.360 --> 00:44:27.097 So I didn't mean to go to the next

NOTE Confidence: 0.959589074

00:44:27.097 --> 00:44:29.297 one so quite so quickly at but you

NOTE Confidence: 0.959589074

00:44:29.297 --> 00:44:31.157 need electron microscopy to see the details.

NOTE Confidence: 0.959589074

00:44:31.160 --> 00:44:34.238 So what Max was able to do was expand

NOTE Confidence: 0.959589074

00:44:34.238 --> 00:44:36.436 primary human platelets and then just

NOTE Confidence: 0.959589074

00:44:36.436 --> 00:44:38.951 this was just the pan staining with

NOTE Confidence: 0.959589074

00:44:38.951 --> 00:44:41.436 the protein stain you can see granules.

NOTE Confidence: 0.959589074

00:44:41.440 --> 00:44:44.920 Then he used antibody against thrombospondin.

NOTE Confidence: 0.959589074

00:44:44.920 --> 00:44:45.186 Oops,

NOTE Confidence: 0.959589074

00:44:45.186 --> 00:44:47.314 it's supposed to be playing Oh well then

NOTE Confidence: 0.959589074

00:44:47.314 --> 00:44:49.437 he used antibiotic and there you go.

NOTE Confidence: 0.959589074

00:44:49.440 --> 00:44:51.080 So sorry.

NOTE Confidence: 0.959589074

00:44:51.080 --> 00:44:52.856 This is the thrombospondin

NOTE Confidence: 0.959589074

00:44:52.856 --> 00:44:55.076 which is in alpha granules.

NOTE Confidence: 0.959589074

00:44:55.080 --> 00:44:57.366 This is staining for tubulin which

NOTE Confidence: 0.959589074

00:44:57.366 --> 00:44:59.919 is on the outside of platelets.

NOTE Confidence: 0.959589074

00:44:59.920 --> 00:45:01.117 So and just the way you expect,

NOTE Confidence: 0.959589074

00:45:01.120 --> 00:45:03.115 we can see this tubulin ring and

NOTE Confidence: 0.959589074

00:45:03.115 --> 00:45:05.350 this is showing you both the tubulin

NOTE Confidence: 0.959589074

00:45:05.350 --> 00:45:07.000 ring and the thrombus bonded.

NOTE Confidence: 0.959589074

00:45:07.000 --> 00:45:09.037 Beautiful. What can we use this for?

NOTE Confidence: 0.959589074

00:45:09.040 --> 00:45:09.255 Well,

NOTE Confidence: 0.959589074

00:45:09.255 --> 00:45:10.975 one of the things we can use it

NOTE Confidence: 0.959589074

00:45:10.975 --> 00:45:13.137 for is to try to quantitate alpha

NOTE Confidence: 0.959589074

00:45:13.137 --> 00:45:14.397 granules within the platelets.

NOTE Confidence: 0.959589074

00:45:14.400 --> 00:45:16.416 And what I'm showing you here on the

NOTE Confidence: 0.959589074

00:45:16.416 --> 00:45:18.644 left is some of the classic work

NOTE Confidence: 0.959589074

00:45:18.644 --> 00:45:20.304 where they were quantifying alpha

NOTE Confidence: 0.959589074

00:45:20.371 --> 00:45:22.261 granules in platelets using electron

NOTE Confidence: 0.959589074

00:45:22.261 --> 00:45:25.336 microscopy and they got about 50 such

NOTE Confidence: 0.959589074

00:45:25.336 --> 00:45:28.276 granules per platelet on average.

NOTE Confidence: 0.959589074

00:45:28.280 --> 00:45:29.568 This is our data,

NOTE Confidence: 0.959589074

00:45:29.568 --> 00:45:31.178 not counting them using electron

NOTE Confidence: 0.959589074

00:45:31.178 --> 00:45:32.801 microscopy where you it's a huge

NOTE Confidence: 0.959589074

00:45:32.801 --> 00:45:35.186 amount of time and effort to try to

NOTE Confidence: 0.959589074

00:45:35.186 --> 00:45:36.634 get this three-dimensional microscopy.

NOTE Confidence: 0.959589074

00:45:36.640 --> 00:45:39.076 Here he can look at 151 platelets

NOTE Confidence: 0.959589074

00:45:39.076 --> 00:45:40.882 in that stained slide that in the

NOTE Confidence: 0.959589074

00:45:40.882 --> 00:45:42.582 slide I just showed you and he can

NOTE Confidence: 0.959589074

00:45:42.582 --> 00:45:44.050 say how many total granules are

NOTE Confidence: 0.959589074

00:45:44.050 --> 00:45:45.954 there and how many granules are there

NOTE Confidence: 0.959589074

00:45:45.960 --> 00:45:47.520 that have thromaspondon in them.

NOTE Confidence: 0.959589074

00:45:47.520 --> 00:45:48.968 And he could see that there were a

NOTE Confidence: 0.959589074

00:45:48.968 --> 00:45:50.307 little bit more than 50 granules

NOTE Confidence: 0.959589074

00:45:50.307 --> 00:45:51.472 on average per platelet looking

NOTE Confidence: 0.959589074

00:45:51.472 --> 00:45:52.400 very similar to this.

NOTE Confidence: 0.959589074

00:45:52.400 --> 00:45:53.814 And then he could even look at

NOTE Confidence: 0.959589074

00:45:53.814 --> 00:45:55.195 what percentage of those platelets

NOTE Confidence: 0.959589074

00:45:55.195 --> 00:45:55.919 have thromaspondon.

NOTE Confidence: 0.959589074

00:45:55.920 --> 00:45:57.384 So again something that I think

NOTE Confidence: 0.959589074

00:45:57.384 --> 00:45:58.360 can be useful clinically,

NOTE Confidence: 0.959589074

00:45:58.360 --> 00:46:02.200 certainly it's interesting scientifically.

NOTE Confidence: 0.959589074

00:46:02.200 --> 00:46:02.808 So finally,

NOTE Confidence: 0.959589074

00:46:02.808 --> 00:46:05.240 this is what I've told you today that

NOTE Confidence: 0.8670297356

00:46:05.307 --> 00:46:07.431 single cell RNA seek reveals MEP

NOTE Confidence: 0.8670297356

00:46:07.431 --> 00:46:09.954 as a unique transitional state in

NOTE Confidence: 0.8670297356

00:46:09.954 --> 00:46:11.520 hematopoietic fate specification.

NOTE Confidence: 0.8670297356

00:46:11.520 --> 00:46:13.220 That cell cycle differences really

NOTE Confidence: 0.8670297356

00:46:13.220 --> 00:46:15.260 seem to regulate MEP fate and

NOTE Confidence: 0.8670297356

00:46:15.260 --> 00:46:16.958 we're trying to figure out how.

NOTE Confidence: 0.8670297356

00:46:16.960 --> 00:46:19.895 One of the ways that seems to be working

NOTE Confidence: 0.8670297356

00:46:19.895 --> 00:46:22.520 is through in a slower cycling cell

NOTE Confidence: 0.8670297356

00:46:22.520 --> 00:46:24.878 there's more phosphoserine Ronx one,

NOTE Confidence: 0.8670297356

00:46:24.880 --> 00:46:26.280 and that phosphoserine Ronx

NOTE Confidence: 0.8670297356

00:46:26.280 --> 00:46:27.680 1 activates Meg genes,

NOTE Confidence: 0.8670297356

00:46:27.680 --> 00:46:30.158 so you get Meg fate specification.

NOTE Confidence: 0.8670297356

00:46:30.160 --> 00:46:32.242 I showed you time lapse imaging

NOTE Confidence: 0.8670297356

00:46:32.242 --> 00:46:34.360 that really showed at least that

NOTE Confidence: 0.8670297356

00:46:34.360 --> 00:46:35.776 statistically significant slowing

NOTE Confidence: 0.8670297356

00:46:35.776 --> 00:46:38.608 of the cell cycle speed predicts

NOTE Confidence: 0.8670297356

00:46:38.608 --> 00:46:40.824 MK fade specification and that

NOTE Confidence: 0.8670297356

00:46:40.824 --> 00:46:42.909 we can predict the probability

NOTE Confidence: 0.8670297356

00:46:42.909 --> 00:46:45.107 of MEP fade specification over

NOTE Confidence: 0.8670297356

00:46:45.107 --> 00:46:47.237 time with this Markov model.

NOTE Confidence: 0.8670297356

00:46:47.240 --> 00:46:49.711 And finally that we're very excited about

NOTE Confidence: 0.8670297356

00:46:49.711 --> 00:46:52.599 the power of using expansion microscopy.

NOTE Confidence: 0.8670297356

00:46:52.600 --> 00:46:54.000 I wanted to take a minute to tell

NOTE Confidence: 0.8670297356

00:46:54.000 --> 00:46:55.306 you about the cooperative centers

NOTE Confidence: 0.8670297356

00:46:55.306 --> 00:46:56.518 of excellence in hematology,

NOTE Confidence: 0.8670297356

00:46:56.520 --> 00:46:57.624 which Karen already mentioned,

NOTE Confidence: 0.8670297356

00:46:57.624 --> 00:46:59.280 but who listens to the intro.

NOTE Confidence: 0.8670297356

00:46:59.280 --> 00:47:04.136 So YCCEH is funded by the NIDDK.

NOTE Confidence: 0.8670297356

00:47:04.136 --> 00:47:06.768 Yale is one of five such centers

NOTE Confidence: 0.8670297356

00:47:06.768 --> 00:47:08.599 nationwide and we all provide

NOTE Confidence: 0.8670297356

00:47:08.599 --> 00:47:10.603 cores that can help people who

NOTE Confidence: 0.8670297356

00:47:10.603 --> 00:47:12.759 do non malignant hematology.

NOTE Confidence: 0.8670297356

00:47:12.760 --> 00:47:14.594 And some of what I showed you

NOTE Confidence: 0.8670297356

00:47:14.594 --> 00:47:16.533 today is available in our core

NOTE Confidence: 0.8670297356

00:47:16.533 --> 00:47:17.997 including the expansion microscopy,

NOTE Confidence: 0.8670297356

00:47:18.000 --> 00:47:20.960 the time lapse microscopy,

NOTE Confidence: 0.8670297356

00:47:20.960 --> 00:47:22.408 CDC's colony forming assays.

NOTE Confidence: 0.8670297356

00:47:22.408 --> 00:47:24.580 We can help you with hematopoietic

NOTE Confidence: 0.8670297356

00:47:24.646 --> 00:47:26.716 assays and other across the country.

NOTE Confidence: 0.8670297356

00:47:26.720 --> 00:47:28.360 There's a metabolomics core for

NOTE Confidence: 0.8670297356

00:47:28.360 --> 00:47:30.000 any non malignant heme work

NOTE Confidence: 0.8670297356

00:47:30.064 --> 00:47:31.678 that you're doing in at Utah.

NOTE Confidence: 0.8670297356

00:47:31.680 --> 00:47:33.735 There's an imaging core that

NOTE Confidence: 0.8670297356

00:47:33.735 --> 00:47:35.428 does codecs in Indiana.

NOTE Confidence: 0.8670297356

00:47:35.428 --> 00:47:38.437 You can get as many CD34 cells as

NOTE Confidence: 0.8670297356

00:47:38.437 --> 00:47:40.111 you would ever need from multiple

NOTE Confidence: 0.8670297356

00:47:40.111 --> 00:47:42.600 types of donors in Seattle,

NOTE Confidence: 0.8670297356

00:47:42.600 --> 00:47:45.210 so do contact me if you want to be part

NOTE Confidence: 0.8670297356

00:47:45.281 --> 00:47:47.751 of that or look it up at cceh dot IO.

NOTE Confidence: 0.8670297356

00:47:47.760 --> 00:47:49.825 Finally, there are grants available

NOTE Confidence: 0.8670297356

00:47:49.825 --> 00:47:51.120 through ICCEH Money,

NOTE Confidence: 0.8670297356

00:47:51.120 --> 00:47:52.440 Money, Money, money.

NOTE Confidence: 0.8670297356

00:47:52.440 --> 00:47:54.195 They have Type A grants and Type B grants.

NOTE Confidence: 0.8670297356

00:47:54.200 --> 00:47:57.522 The Type A grants give you $12,000 worth

NOTE Confidence: 0.8670297356

00:47:57.522 --> 00:48:01.320 of services at any one of the five cores,

NOTE Confidence: 0.8670297356

00:48:01.320 --> 00:48:03.959 and those are it's a rolling submission.

NOTE Confidence: 0.8670297356

00:48:03.960 --> 00:48:05.864 Anytime you have one of these just

NOTE Confidence: 0.8670297356

00:48:05.864 --> 00:48:07.666 submit it and we'll we review

NOTE Confidence: 0.8670297356

00:48:07.666 --> 00:48:09.184 the monthly and then the Type

NOTE Confidence: 0.8670297356

00:48:09.184 --> 00:48:10.656 B grants are up to 70,000.

NOTE Confidence: 0.8670297356

00:48:10.656 --> 00:48:13.552 They take an 8% overhead out of that

NOTE Confidence: 0.8670297356

00:48:13.552 --> 00:48:15.880 $70,000 for you for your research

NOTE Confidence: 0.8670297356

00:48:15.880 --> 00:48:17.612 for non malignant hematology.

NOTE Confidence: 0.8670297356

00:48:17.612 --> 00:48:20.744 And those Type B grants are due

NOTE Confidence: 0.8670297356

00:48:20.744 --> 00:48:23.285 February 15th I think don't quote me

NOTE Confidence: 0.8670297356

00:48:23.285 --> 00:48:25.718 on that go to I go to CCH dot IO.

NOTE Confidence: 0.8670297356

00:48:25.720 --> 00:48:28.436 But really it's it's they're good grants.

NOTE Confidence: 0.8670297356

00:48:28.440 --> 00:48:32.840 So finally thank you to the lab,

NOTE Confidence: 0.8670297356

00:48:32.840 --> 00:48:34.250 everybody's pictured here and hopefully

NOTE Confidence: 0.8670297356

00:48:34.250 --> 00:48:36.360 I gave them credit as we went along.

NOTE Confidence: 0.8670297356

00:48:36.360 --> 00:48:36.960 Thanks so much.

NOTE Confidence: 0.666180874

00:48:45.640 --> 00:48:46.640 This is open for questions.

NOTE Confidence: 0.5525023

00:48:50.480 --> 00:48:50.720 Yeah,

NOTE Confidence: 0.2580808375

00:48:52.800 --> 00:48:55.680 expansion by class is 34.

NOTE Confidence: 0.2580808375

00:48:55.680 --> 00:48:58.765 I'm wondering do you know if

NOTE Confidence: 0.2580808375

00:48:58.765 --> 00:49:00.529 that expansion material disrupt

NOTE Confidence: 0.2580808375

00:49:00.529 --> 00:49:02.999 like protein public interaction,

NOTE Confidence: 0.2580808375

00:49:03.000 --> 00:49:04.792 did you try to see that colonization

NOTE Confidence: 0.2580808375

00:49:04.792 --> 00:49:07.480 of sort of things? Yeah. So

NOTE Confidence: 0.707747023333333

00:49:10.640 --> 00:49:12.398 the answer is that proteins stay

NOTE Confidence: 0.707747023333333

00:49:12.400 --> 00:49:14.564 intact and protein interactions they

NOTE Confidence: 0.707747023333333

00:49:14.564 --> 00:49:16.520 they're they say look Co localized,

NOTE Confidence: 0.707747023333333

00:49:16.520 --> 00:49:18.424 but I don't know if they stay

NOTE Confidence: 0.707747023333333

00:49:18.424 --> 00:49:20.120 negative if it's not prevailed.

NOTE Confidence: 0.707747023333333

00:49:20.120 --> 00:49:23.272 What you what you can do though with

NOTE Confidence: 0.707747023333333

00:49:23.272 --> 00:49:25.399 extended microscopy is Co localized

NOTE Confidence: 0.707747023333333

00:49:25.399 --> 00:49:27.931 2 proteins that you cannot clearly

NOTE Confidence: 0.707747023333333

00:49:27.931 --> 00:49:30.398 visualize if you don't have extension.

NOTE Confidence: 0.707747023333333

00:49:30.400 --> 00:49:32.640 So if you stain them after you've extended,

NOTE Confidence: 0.707747023333333

00:49:32.640 --> 00:49:34.170 you'll really be able to see

NOTE Confidence: 0.707747023333333

00:49:34.170 --> 00:49:35.938 that they were right next to each

NOTE Confidence: 0.707747023333333

00:49:35.938 --> 00:49:37.905 other and all of the epitopes will

NOTE Confidence: 0.707747023333333

00:49:37.905 --> 00:49:39.772 still be there because they're not

NOTE Confidence: 0.707747023333333

00:49:39.772 --> 00:49:41.560 blocking one another by being bad.

NOTE Confidence: 0.707747023333333

00:49:41.560 --> 00:49:43.840 So people have done Co localization

NOTE Confidence: 0.707747023333333

00:49:43.840 --> 00:49:45.800 studies with expansion that weren't

NOTE Confidence: 0.707747023333333

00:49:45.800 --> 00:49:47.950 feasible prior to having expansion.

NOTE Confidence: 0.707747023333333

00:49:47.950 --> 00:49:50.715 But if you're asking other things when we

NOTE Confidence: 0.707747023333333

00:49:50.715 --> 00:49:53.079 don't know what happens to DNA and RNA,

NOTE Confidence: 0.707747023333333

00:49:53.080 --> 00:49:55.117 some people have gotten fish to work,

NOTE Confidence: 0.707747023333333

00:49:55.120 --> 00:49:57.136 but I don't really know what the

NOTE Confidence: 0.707747023333333

00:49:57.136 --> 00:49:59.104 stretching does and what exactly gets

NOTE Confidence: 0.707747023333333

00:49:59.104 --> 00:50:01.426 stretched at that tiny molecular level.

NOTE Confidence: 0.707747023333333

00:50:01.426 --> 00:50:03.920 I've asked the same questions to the answer,

NOTE Confidence: 0.707747023333333

00:50:03.920 --> 00:50:05.120 but I'm not sure you know,

NOTE Confidence: 0.5280967575

00:50:06.600 --> 00:50:09.720 with your increase in drugs on

NOTE Confidence: 0.5280967575

00:50:09.720 --> 00:50:13.478 causing the increase in accuracy.

NOTE Confidence: 0.5280967575

00:50:13.480 --> 00:50:16.680 Do you know that this later gets rise

NOTE Confidence: 0.5280967575

00:50:16.680 --> 00:50:18.560 to functional increase in platelets?

NOTE Confidence: 0.749041935

00:50:20.880 --> 00:50:23.348 No. But what we do know,

NOTE Confidence: 0.749041935

00:50:23.348 --> 00:50:24.770 so our work was unique in

NOTE Confidence: 0.807950995517242

00:50:24.829 --> 00:50:26.549 starting with the bipodent progenitor

NOTE Confidence: 0.807950995517242

00:50:26.549 --> 00:50:28.600 and what we were always looking

NOTE Confidence: 0.807950995517242

00:50:28.600 --> 00:50:30.752 for is just which fake did it pick.

NOTE Confidence: 0.807950995517242

00:50:30.760 --> 00:50:32.344 And so you're right, we're only

NOTE Confidence: 0.807950995517242

00:50:32.344 --> 00:50:35.200 looking like the first part of it,

NOTE Confidence: 0.807950995517242

00:50:35.200 --> 00:50:36.130 but we didn't come up with

NOTE Confidence: 0.807950995517242

00:50:36.130 --> 00:50:37.880 rocks all by ourselves.

NOTE Confidence: 0.807950995517242

00:50:37.880 --> 00:50:40.078 Bronx One is known in a mouse.

NOTE Confidence: 0.807950995517242

00:50:40.080 --> 00:50:41.358 If you knock down Bronx One,

NOTE Confidence: 0.807950995517242

00:50:41.360 --> 00:50:43.436 you have lower meds, lower platelets.

NOTE Confidence: 0.807950995517242

00:50:43.440 --> 00:50:44.560 If you over fresh rocks,

NOTE Confidence: 0.807950995517242

00:50:44.560 --> 00:50:46.240 you have more meds and more platelets.

NOTE Confidence: 0.807950995517242

00:50:46.240 --> 00:50:47.902 What wasn't known is where was

NOTE Confidence: 0.807950995517242

00:50:47.902 --> 00:50:49.714 that acting and that it might

NOTE Confidence: 0.807950995517242

00:50:49.714 --> 00:50:51.664 be acting literally at the Fate

NOTE Confidence: 0.807950995517242

00:50:51.664 --> 00:50:52.920 specification level of an MEP.

NOTE Confidence: 0.807950995517242

00:50:52.920 --> 00:50:54.840 So I think they would,

NOTE Confidence: 0.807950995517242

00:50:54.840 --> 00:50:55.995 but I can't tell you for sure.

NOTE Confidence: 0.4893077

00:50:56.400 --> 00:50:58.066 I ask this because of our patients

NOTE Confidence: 0.4893077

00:50:58.066 --> 00:51:01.600 with rocks one journal on mutation

NOTE Confidence: 0.4893077

00:51:01.600 --> 00:51:04.480 and they in the bone marrow have the

NOTE Confidence: 0.4893077

00:51:04.480 --> 00:51:07.000 creation of abnormal and carrying sites,

NOTE Confidence: 0.4893077

00:51:07.000 --> 00:51:08.398 but then they have Bronx therapy.

NOTE Confidence: 0.73607370875

00:51:09.720 --> 00:51:13.040 Those patients actually are

NOTE Confidence: 0.73607370875

00:51:13.040 --> 00:51:16.360 hemisitis for inactivating mutation.

NOTE Confidence: 0.73607370875

00:51:16.360 --> 00:51:20.040 They have decreased Bronx activity.

NOTE Confidence: 0.73607370875

00:51:20.040 --> 00:51:22.630 So their mutant Bronx is either hypo

NOTE Confidence: 0.73607370875

00:51:22.630 --> 00:51:25.240 functioning or not functioning at all.

NOTE Confidence: 0.73607370875

00:51:25.240 --> 00:51:27.760 I wasn't aware then more plate, more megs.

NOTE Confidence: 0.73607370875

00:51:27.760 --> 00:51:30.096 I know they have lower ploying megs because

NOTE Confidence: 0.73607370875

00:51:30.096 --> 00:51:32.307 they have a defect in Meg maturation

NOTE Confidence: 0.73607370875

00:51:32.307 --> 00:51:34.000 and they have lower platelets. Yeah,

NOTE Confidence: 0.73544616

00:51:36.960 --> 00:51:39.536 yes, yes, it's really beautiful.

NOTE Confidence: 0.73544616

00:51:39.536 --> 00:51:41.840 I was wondering that the the

NOTE Confidence: 0.524295674615385

00:51:41.917 --> 00:51:44.407 the common progenitor and in vitro,

NOTE Confidence: 0.524295674615385

00:51:44.407 --> 00:51:46.501 the lineage commitment from the Detroit

NOTE Confidence: 0.524295674615385

00:51:46.501 --> 00:51:49.048 and and Medicare is obviously driven by

NOTE Confidence: 0.524295674615385

00:51:49.048 --> 00:51:51.519 the cell cycle towards that it's right.

NOTE Confidence: 0.524295674615385

00:51:51.520 --> 00:51:54.790 Could you understand your situation in

NOTE Confidence: 0.524295674615385

00:51:54.790 --> 00:51:57.730 vivo where those things are altered in

NOTE Confidence: 0.524295674615385

00:51:57.730 --> 00:52:00.608 a way that the the ratio you know the

NOTE Confidence: 0.524295674615385

00:52:00.608 --> 00:52:01.952 cell has to decide it's going to make?

NOTE Confidence: 0.524295674615385

00:52:01.960 --> 00:52:04.676 How many RPCS and how many pavements?

NOTE Confidence: 0.524295674615385

00:52:04.680 --> 00:52:07.277 In which situation does it go awry

NOTE Confidence: 0.524295674615385

00:52:07.280 --> 00:52:11.600 and is it truly lineage commitment

NOTE Confidence: 0.524295674615385

00:52:11.600 --> 00:52:13.480 between or is it just stochastic? Well,

NOTE Confidence: 0.820013413333333

00:52:14.560 --> 00:52:16.360 we think it's truly lineage commitment

NOTE Confidence: 0.53965566

00:52:17.440 --> 00:52:19.040 on a stochastic low because

NOTE Confidence: 0.807673904545455

00:52:19.040 --> 00:52:20.021 there's always probability.

NOTE Confidence: 0.807673904545455

00:52:20.021 --> 00:52:22.680 We don't see that if we overspress rocks,

NOTE Confidence: 0.807673904545455

00:52:22.680 --> 00:52:23.556 everything goes in it.

NOTE Confidence: 0.807673904545455

00:52:23.556 --> 00:52:25.480 It's just some ability to go overthrow it.

NOTE Confidence: 0.807673904545455

00:52:25.480 --> 00:52:27.664 In fact you need rocks one

NOTE Confidence: 0.807673904545455

00:52:27.664 --> 00:52:28.756 for erythroid maturation.

NOTE Confidence: 0.807673904545455

00:52:28.760 --> 00:52:32.197 So I think it's stochastic but biased

NOTE Confidence: 0.807673904545455

00:52:32.200 --> 00:52:34.195 that you know you have one ratio.

NOTE Confidence: 0.807673904545455

00:52:34.200 --> 00:52:35.604 In the absence of over expressing

NOTE Confidence: 0.807673904545455

00:52:35.604 --> 00:52:37.396 wrongs you get a ratio that's very

NOTE Confidence: 0.807673904545455

00:52:37.396 --> 00:52:38.998 Meg biased when you over express

NOTE Confidence: 0.807673904545455

00:52:38.998 --> 00:52:40.676 wrongs and more Meg bias if you have

NOTE Confidence: 0.737836598

00:52:40.920 --> 00:52:42.960 normal. But in a normal progenitor

NOTE Confidence: 0.737836598

00:52:42.960 --> 00:52:44.804 what is the ratio of commitment

NOTE Confidence: 0.737836598

00:52:44.804 --> 00:52:46.014 towards something like a carrier

NOTE Confidence: 0.737836598

00:52:46.014 --> 00:52:47.440 site and the unit for itself?

NOTE Confidence: 0.678241175

00:52:47.800 --> 00:52:49.494 You're asking in vivo and I can

NOTE Confidence: 0.678241175

00:52:49.494 --> 00:52:51.180 only tell you in vitro, yeah,

NOTE Confidence: 0.678241175

00:52:51.180 --> 00:52:53.096 or even in vitro. In vitro,

NOTE Confidence: 0.678241175

00:52:53.096 --> 00:52:56.440 it seems that they're about equal and

NOTE Confidence: 0.678241175

00:52:56.440 --> 00:52:58.680 there's a there's a good reason for that.

NOTE Confidence: 0.678241175

00:52:58.680 --> 00:53:01.230 What happens downstream is the

NOTE Confidence: 0.678241175

00:53:01.230 --> 00:53:02.760 erythroid progenitor proliferates

NOTE Confidence: 0.678241175

00:53:02.760 --> 00:53:05.363 log fold multiple times very quickly

NOTE Confidence: 0.678241175

00:53:05.363 --> 00:53:08.120 to make a lot of erythroid cells.

NOTE Confidence: 0.678241175

00:53:08.120 --> 00:53:09.980 The Meg progenitor doesn't

NOTE Confidence: 0.678241175

00:53:09.980 --> 00:53:11.840 proliferate very many times,

NOTE Confidence: 0.678241175

00:53:11.840 --> 00:53:13.910 but each mega carry site makes

NOTE Confidence: 0.678241175

00:53:13.910 --> 00:53:16.120 10 to the three platelets.

NOTE Confidence: 0.678241175

00:53:16.120 --> 00:53:19.540 So you have a three log production

NOTE Confidence: 0.678241175

00:53:19.540 --> 00:53:21.210 per mega Carrison.

NOTE Confidence: 0.678241175

00:53:21.210 --> 00:53:23.520 So it kind of works mathematically.

NOTE Confidence: 0.678241175

00:53:23.520 --> 00:53:24.948 If you say you make one play

NOTE Confidence: 0.678241175

00:53:24.948 --> 00:53:26.725 then one or it's all about you

NOTE Confidence: 0.678241175

00:53:26.725 --> 00:53:27.715 know 1 to 1 ratios that

NOTE Confidence: 0.891264846

00:53:27.720 --> 00:53:29.000 that's how it would go. And

NOTE Confidence: 0.508137418181818

00:53:29.000 --> 00:53:31.667 we know if in older adults where

NOTE Confidence: 0.508137418181818

00:53:31.667 --> 00:53:33.600 there is minority buys that

NOTE Confidence: 0.508137418181818

00:53:33.600 --> 00:53:35.906 that there is a a differential

NOTE Confidence: 0.508137418181818

00:53:35.906 --> 00:53:37.671 response to this commitment between

NOTE Confidence: 0.508137418181818

00:53:37.671 --> 00:53:39.280 megataryocytes and heart disease. I

NOTE Confidence: 0.90591115631579

00:53:39.280 --> 00:53:41.624 don't know, I'd love to actually get access

NOTE Confidence: 0.90591115631579

00:53:41.624 --> 00:53:43.888 to marrow from patients with different

NOTE Confidence: 0.90591115631579

00:53:43.888 --> 00:53:45.913 diseases and that's been problematic.

NOTE Confidence: 0.90591115631579

00:53:45.920 --> 00:53:50.130 We have looked at MPNS and in MPNS if they

NOTE Confidence: 0.90591115631579

00:53:50.238 --> 00:53:51.944 have essential thrombocytosis then they

NOTE Confidence: 0.90591115631579

00:53:51.944 --> 00:53:55.288 do tend to have a Meg bias to their MEP

NOTE Confidence: 0.90591115631579

00:53:55.288 --> 00:53:57.280 and the opposite for polysychemia Vera.

NOTE Confidence: 0.90591115631579

00:53:57.280 --> 00:53:58.448 But it's very subtle.

NOTE Confidence: 0.90591115631579

00:53:58.448 --> 00:54:01.444 I think a lot of that is downstream of

NOTE Confidence: 0.90591115631579

00:54:01.444 --> 00:54:03.796 the MEP and the FATE certification.

NOTE Confidence: 0.90591115631579

00:54:03.800 --> 00:54:06.862 Jack 2:00 and from and Teepo, they're there.

NOTE Confidence: 0.90591115631579

00:54:06.862 --> 00:54:08.517 They're acted the whole time.

NOTE Confidence: 0.90591115631579

00:54:08.520 --> 00:54:09.997 So it's not going to just toggle

NOTE Confidence: 0.668145923333333

00:54:10.000 --> 00:54:11.800 it. Yeah. So

NOTE Confidence: 0.664692644

00:54:11.800 --> 00:54:13.720 along those lines that you looked or

NOTE Confidence: 0.664692644

00:54:13.720 --> 00:54:17.200 what do you know about CHIP and actually

NOTE Confidence: 0.595743156363636

00:54:17.600 --> 00:54:18.401 mutations in Ronczuan,

NOTE Confidence: 0.595743156363636

00:54:18.401 --> 00:54:20.600 a lot of things in terms of their

NOTE Confidence: 0.595743156363636

00:54:20.600 --> 00:54:22.880 cell cycling and their biases,

NOTE Confidence: 0.694387464117647

00:54:23.080 --> 00:54:25.984 nothing but. But patients with Ronczuan

NOTE Confidence: 0.694387464117647

00:54:25.984 --> 00:54:27.920 familial mutations in Ronczuan

NOTE Confidence: 0.694387464117647

00:54:27.995 --> 00:54:30.277 do have an increased risk of Chip

NOTE Confidence: 0.688225391

00:54:32.800 --> 00:54:34.408 that might just be because they

NOTE Confidence: 0.688225391

00:54:34.408 --> 00:54:35.480 have abnormal Hemato policies.

NOTE Confidence: 0.688225391

00:54:35.480 --> 00:54:37.223 And so the few, the better cells

NOTE Confidence: 0.688225391

00:54:37.223 --> 00:54:39.198 are the ones that are taking over.

NOTE Confidence: 0.688225391

00:54:39.200 --> 00:54:40.355 But I I don't know for sure.

NOTE Confidence: 0.688225391

00:54:40.360 --> 00:54:42.040 It's a good question,

NOTE Confidence: 0.688225391

00:54:42.040 --> 00:54:42.576 really good question.

NOTE Confidence: 0.688225391

00:54:42.576 --> 00:54:45.011 It'll be fun to look at that.

NOTE Confidence: 0.688225391

00:54:45.011 --> 00:54:46.488 We have a lot of such patients

NOTE Confidence: 0.688225391

00:54:46.488 --> 00:54:48.120 that we can get access to cells.

NOTE Confidence: 0.36197081

00:54:52.880 --> 00:54:53.560 That question

NOTE Confidence: 0.52111016

00:54:55.640 --> 00:54:57.188 hypothetically speaking, eventually

NOTE Confidence: 0.52111016

00:54:57.188 --> 00:55:00.800 the red blood cells will be Euclided.

NOTE Confidence: 0.52111016

00:55:00.800 --> 00:55:02.736 Is there part of the process that they

NOTE Confidence: 0.52111016

00:55:02.736 --> 00:55:04.825 don't have to have a nucleus in the end

NOTE Confidence: 0.52111016

00:55:04.825 --> 00:55:06.680 that allows them to proliferate so fast?

NOTE Confidence: 0.52111016

00:55:06.680 --> 00:55:09.508 Is the ability of proliferation is is

NOTE Confidence: 0.52111016

00:55:09.508 --> 00:55:11.877 reduced because they don't have to

NOTE Confidence: 0.52111016

00:55:11.877 --> 00:55:13.716 maintain the full sort of nucleus.

NOTE Confidence: 0.52111016

00:55:13.716 --> 00:55:15.480 They can just go faster by being

NOTE Confidence: 0.52111016

00:55:15.531 --> 00:55:16.916 more efficient in that way.

NOTE Confidence: 0.52111016

00:55:16.920 --> 00:55:17.120 They keep

NOTE Confidence: 0.427930538

00:55:17.200 --> 00:55:19.040 it absolutely as well they're.

NOTE Confidence: 0.618049331428571

00:55:19.520 --> 00:55:20.920 But content is the content the same?

NOTE Confidence: 0.618049331428571

00:55:20.920 --> 00:55:23.116 Do you know what the size

NOTE Confidence: 0.618049331428571

00:55:23.120 --> 00:55:24.236 as they as they go forward?

NOTE Confidence: 0.484321094285714

00:55:24.240 --> 00:55:26.039 It's a good question for PAD Gallup.

NOTE Confidence: 0.484321094285714

00:55:26.040 --> 00:55:30.520 Here we go. What we do know is that

NOTE Confidence: 0.484321094285714

00:55:30.520 --> 00:55:33.480 as these erythroid cells are matured,

NOTE Confidence: 0.484321094285714

00:55:33.480 --> 00:55:34.440 they're proliferating.

NOTE Confidence: 0.484321094285714

00:55:34.440 --> 00:55:37.320 Again, matured that with the maturation,

NOTE Confidence: 0.484321094285714

00:55:37.320 --> 00:55:39.600 the nucleus shuts down and the histones

NOTE Confidence: 0.484321094285714

00:55:39.600 --> 00:55:42.320 get spit out. But prior to that,

NOTE Confidence: 0.484321094285714

00:55:42.320 --> 00:55:43.920 when they're so proliferating,

NOTE Confidence: 0.484321094285714

00:55:43.920 --> 00:55:46.125 I'm not aware of what's changing at

NOTE Confidence: 0.484321094285714

00:55:46.125 --> 00:55:47.960 the chromatin level, but correct.

NOTE Confidence: 0.484321094285714

00:55:47.960 --> 00:55:49.308 But that's been published.

NOTE Confidence: 0.484321094285714

00:55:49.308 --> 00:55:50.319 I should know.

NOTE Confidence: 0.484321094285714

00:55:50.320 --> 00:55:52.920 It's because Pat's published,

NOTE Confidence: 0.484321094285714

00:55:52.920 --> 00:55:53.832 I think what they,

NOTE Confidence: 0.484321094285714

00:55:53.832 --> 00:55:55.200 I think if I remember correctly,

NOTE Confidence: 0.484321094285714

00:55:55.200 --> 00:55:56.970 they express fewer and fewer genes

NOTE Confidence: 0.484321094285714

00:55:56.970 --> 00:55:58.776 and higher and higher levels of

NOTE Confidence: 0.484321094285714

00:55:58.776 --> 00:56:00.474 the erythroid genes and you know,

NOTE Confidence: 0.484321094285714

00:56:00.480 --> 00:56:01.615 like globins because it's going

NOTE Confidence: 0.484321094285714

00:56:01.615 --> 00:56:02.995 to need all that globin for

NOTE Confidence: 0.484321094285714

00:56:02.995 --> 00:56:04.195 when it doesn't have a nucleus

NOTE Confidence: 0.59440742

00:56:04.240 --> 00:56:05.900 if they give them a timing advantage, if

NOTE Confidence: 0.59440742

00:56:05.900 --> 00:56:07.520 they're going to that moment. So they can. I

NOTE Confidence: 0.597231438888889

00:56:08.080 --> 00:56:09.490 don't know why it sounds like

NOTE Confidence: 0.597231438888889

00:56:09.490 --> 00:56:10.760 being so fast. It's part of their

NOTE Confidence: 0.291903385

00:56:12.240 --> 00:56:16.600 own. Yeah, throughout that. So you can see,

NOTE Confidence: 0.850209194814815

00:56:17.680 --> 00:56:19.451 so can you see any advantage to

NOTE Confidence: 0.850209194814815

00:56:19.451 --> 00:56:21.314 cycling faster or that you can cycle

NOTE Confidence: 0.850209194814815

00:56:21.314 --> 00:56:23.233 faster because you don't need so much

NOTE Confidence: 0.850209194814815

00:56:23.233 --> 00:56:24.955 activity going on in your nucleus,

NOTE Confidence: 0.28122279

00:56:29.320 --> 00:56:32.280 which is like slow down. Yeah,

NOTE Confidence: 0.456336573333333

00:56:35.000 --> 00:56:36.116 sticking outside. Oh, I like it,

NOTE Confidence: 0.718126226153846

00:56:36.160 --> 00:56:37.880 I like it. Let me know when you

NOTE Confidence: 0.718126226153846

00:56:37.880 --> 00:56:40.080 have to go to that conference.

NOTE Confidence: 0.48513156

00:56:40.440 --> 00:56:41.950 So in addition to intrinsic

NOTE Confidence: 0.48513156

00:56:41.950 --> 00:56:43.760 things that would be

NOTE Confidence: 0.44606757

00:56:45.840 --> 00:56:48.000 differentiate, you can see downstream

NOTE Confidence: 0.44606757

00:56:48.000 --> 00:56:49.666 what about contribution from other

NOTE Confidence: 0.44606757

00:56:49.666 --> 00:56:51.515 cell types either from other chromatic

NOTE Confidence: 0.44606757

00:56:51.515 --> 00:56:53.480 cells and signals or thrombo cells.

NOTE Confidence: 0.831722017083333

00:56:55.720 --> 00:56:58.200 We have looked really hard for other parts

NOTE Confidence: 0.831722017083333

00:56:58.200 --> 00:57:00.602 of the micro environment that might affect

NOTE Confidence: 0.831722017083333

00:57:00.602 --> 00:57:03.837 MEP fate and I did not include those data,

NOTE Confidence: 0.831722017083333

00:57:03.840 --> 00:57:05.775 but we've done a lot of work and and

NOTE Confidence: 0.831722017083333

00:57:05.775 --> 00:57:07.360 Vanessa's published quite a bit on it.

NOTE Confidence: 0.831722017083333

00:57:07.360 --> 00:57:09.961 One thing we know is there are two growth

NOTE Confidence: 0.831722017083333

00:57:09.961 --> 00:57:12.383 factors that may many of you may be

NOTE Confidence: 0.831722017083333

00:57:12.383 --> 00:57:14.399 aware of erythropodent and thrombopodent.

NOTE Confidence: 0.831722017083333

00:57:14.400 --> 00:57:16.230 Thrombopodin sounds like it's making

NOTE Confidence: 0.831722017083333

00:57:16.230 --> 00:57:17.490 platelets, right, Thrombopodin,

NOTE Confidence: 0.831722017083333

00:57:17.490 --> 00:57:19.270 erythropodin making erythroid but

NOTE Confidence: 0.831722017083333

00:57:19.270 --> 00:57:21.552 they actually act super differently

NOTE Confidence: 0.831722017083333

00:57:21.552 --> 00:57:22.878 on different cells.

NOTE Confidence: 0.831722017083333

00:57:22.880 --> 00:57:24.748 Thrombopodin is the thrombopodin

NOTE Confidence: 0.831722017083333

00:57:24.748 --> 00:57:27.550 receptor is is on hematopoietic stem

NOTE Confidence: 0.831722017083333

00:57:27.618 --> 00:57:29.916 cell and all of those progenitors.

NOTE Confidence: 0.831722017083333

00:57:29.920 --> 00:57:31.400 So they all need thrombopodin

NOTE Confidence: 0.831722017083333

00:57:31.400 --> 00:57:33.270 and they're it's binding in the

NOTE Confidence: 0.831722017083333

00:57:33.270 --> 00:57:34.915 middle of the thrombopod receptor.

NOTE Confidence: 0.831722017083333

00:57:34.920 --> 00:57:37.373 When you get to the anti P level though,

NOTE Confidence: 0.831722017083333

00:57:37.373 --> 00:57:42.072 the erythroid progenitor loses its erythroid,

NOTE Confidence: 0.831722017083333

00:57:42.072 --> 00:57:44.316 its thrombopodin receptor,

NOTE Confidence: 0.831722017083333

00:57:44.320 --> 00:57:47.146 so it does not have ****** on it,

NOTE Confidence: 0.831722017083333

00:57:47.146 --> 00:57:50.597 and the MEP progenitor has increased ******.

NOTE Confidence: 0.831722017083333

00:57:50.600 --> 00:57:52.460 What we thought then is if we add

NOTE Confidence: 0.831722017083333

00:57:52.460 --> 00:57:54.255 ****** or remove ****** we're going

NOTE Confidence: 0.831722017083333

00:57:54.255 --> 00:57:55.515 to affect faith specification.

NOTE Confidence: 0.831722017083333

00:57:55.520 --> 00:57:57.770 No, what happened is when you

NOTE Confidence: 0.831722017083333

00:57:57.770 --> 00:57:58.520 remove thrombocodin,

NOTE Confidence: 0.831722017083333

00:57:58.520 --> 00:57:59.600 I'm sorry saying that one.

NOTE Confidence: 0.831722017083333

00:57:59.600 --> 00:58:02.168 When you remove thrombocodin you get

NOTE Confidence: 0.831722017083333

00:58:02.168 --> 00:58:05.119 exactly the same ratio of colony types,

NOTE Confidence: 0.831722017083333

00:58:05.120 --> 00:58:07.520 but way fewer colonies and the

NOTE Confidence: 0.831722017083333

00:58:07.520 --> 00:58:09.120 colonies are teeny tiny.

NOTE Confidence: 0.831722017083333

00:58:09.120 --> 00:58:10.737 And when we look at the time

NOTE Confidence: 0.831722017083333

00:58:10.737 --> 00:58:11.720 lapse microscopy of that,

NOTE Confidence: 0.831722017083333

00:58:11.720 --> 00:58:14.114 what we see is that the cells are dying.

NOTE Confidence: 0.831722017083333

00:58:14.120 --> 00:58:15.244 So they're trying to,

NOTE Confidence: 0.831722017083333

00:58:15.244 --> 00:58:16.368 they're doing everything right

NOTE Confidence: 0.831722017083333

00:58:16.368 --> 00:58:18.072 at the beginning and then you

NOTE Confidence: 0.831722017083333

00:58:18.072 --> 00:58:19.000 can just see apoptosis.

NOTE Confidence: 0.831722017083333

00:58:19.000 --> 00:58:19.597 I don't know,

NOTE Confidence: 0.831722017083333

00:58:19.597 --> 00:58:21.248 I didn't prove it was after you see

NOTE Confidence: 0.831722017083333

00:58:21.248 --> 00:58:23.110 the cells dying with erythropoietin,

NOTE Confidence: 0.831722017083333

00:58:23.110 --> 00:58:26.800 again no difference in fate specification,

NOTE Confidence: 0.831722017083333

00:58:26.800 --> 00:58:29.956 but a lack of erythroid maturation,

NOTE Confidence: 0.831722017083333

00:58:29.960 --> 00:58:30.659 absolutely no difference

NOTE Confidence: 0.831722017083333

00:58:30.659 --> 00:58:32.532 in the ratio of output.

NOTE Confidence: 0.831722017083333

00:58:32.532 --> 00:58:35.213 We have seen an effect in this and

NOTE Confidence: 0.831722017083333

00:58:35.213 --> 00:58:36.996 this is part of Vanessa Scanlon's

NOTE Confidence: 0.831722017083333

00:58:36.996 --> 00:58:40.152 work now in the lab that when she Co

NOTE Confidence: 0.831722017083333

00:58:40.152 --> 00:58:42.072 cultures the cells with endothelial

NOTE Confidence: 0.831722017083333

00:58:42.072 --> 00:58:45.040 cells then she also sees an an

NOTE Confidence: 0.831722017083333

00:58:45.040 --> 00:58:46.399 erythroid phase specification.

NOTE Confidence: 0.831722017083333

00:58:46.400 --> 00:58:48.450 It's it's subtle but she can

NOTE Confidence: 0.831722017083333

00:58:48.450 --> 00:58:49.750 see a statistically significant

NOTE Confidence: 0.831722017083333

00:58:49.750 --> 00:58:51.880 increase in E phase specification.

NOTE Confidence: 0.831722017083333

00:58:51.880 --> 00:58:54.152 What she wants to do now is very

NOTE Confidence: 0.831722017083333

00:58:54.152 --> 00:58:55.729 methodically add different cell types

NOTE Confidence: 0.831722017083333

00:58:55.729 --> 00:58:57.927 that are in the bone marrow micro

NOTE Confidence: 0.831722017083333

00:58:57.985 --> 00:59:00.703 environment and determine how they affect

NOTE Confidence: 0.831722017083333

00:59:00.703 --> 00:59:02.515 NDP phase specification individually

NOTE Confidence: 0.831722017083333

00:59:02.520 --> 00:59:04.758 and together and then determine how.

NOTE Confidence: 0.831722017083333

00:59:04.760 --> 00:59:05.796 But that's as much as we know,

NOTE Confidence: 0.831722017083333

00:59:05.800 --> 00:59:07.354 but it's definitely not TECO and ECO.

NOTE Confidence: 0.4187528975

00:59:08.640 --> 00:59:10.400 Has she tried macrophages

NOTE Confidence: 0.4187528975

00:59:10.400 --> 00:59:12.800 since there's the, you know,

NOTE Confidence: 0.2188043

00:59:15.960 --> 00:59:17.745 I think she did once but

NOTE Confidence: 0.2188043

00:59:17.745 --> 00:59:19.330 she didn't have really good

NOTE Confidence: 0.897041930769231

00:59:19.395 --> 00:59:20.598 macrophages to use.

NOTE Confidence: 0.897041930769231

00:59:20.600 --> 00:59:22.686 We're much better in my lab at

NOTE Confidence: 0.897041930769231

00:59:22.686 --> 00:59:24.240 making urine macrophages than human.

NOTE Confidence: 0.897041930769231

00:59:24.240 --> 00:59:26.240 So I'd say we haven't done that adequately.

NOTE Confidence: 0.586639343333333

00:59:28.120 --> 00:59:28.678 I am here,

NOTE Confidence: 0.503246048571429

00:59:29.720 --> 00:59:31.435 very nice talk. So I think this

NOTE Confidence: 0.53200364

00:59:31.440 --> 00:59:31.878 is just a

NOTE Confidence: 0.459704265

00:59:36.800 --> 00:59:40.920 using the IPS cell, it's with the

NOTE Confidence: 0.48968234

00:59:40.920 --> 00:59:42.439 fringe and all kind of other cells,

NOTE Confidence: 0.48968234

00:59:42.440 --> 00:59:43.840 but they cannot differentiate

NOTE Confidence: 0.48968234

00:59:43.840 --> 00:59:45.238 that character. Is that true?

NOTE Confidence: 0.569767385

00:59:46.560 --> 00:59:48.200 They can make mix.

NOTE Confidence: 0.569767385

00:59:48.200 --> 00:59:50.434 They can make mix and in fact there's

NOTE Confidence: 0.569767385

00:59:50.434 --> 00:59:52.640 even one really good scientist who

NOTE Confidence: 0.569767385

00:59:52.640 --> 00:59:56.240 has made IPS derived megacarius like

NOTE Confidence: 0.569767385

00:59:56.240 --> 00:59:59.400 progenitor cell line that is a it's a

NOTE Confidence: 0.569767385

00:59:59.400 --> 01:00:00.640 really beautiful model for studying.

NOTE Confidence: 0.569767385

01:00:00.640 --> 01:00:03.146 You know you can get different mutations

NOTE Confidence: 0.569767385

01:00:03.146 --> 01:00:05.788 from the patients make IPSC make this

NOTE Confidence: 0.569767385

01:00:05.788 --> 01:00:08.920 Meg cell line and basically it doesn't

NOTE Confidence: 0.569767385

01:00:08.920 --> 01:00:11.200 look renty until you then induce

NOTE Confidence: 0.464620721428571

01:00:12.520 --> 01:00:16.720 the movie show. When you see that, terrified,

NOTE Confidence: 0.640357305

01:00:18.840 --> 01:00:21.720 do you see that? The size,

NOTE Confidence: 0.640357305

01:00:21.720 --> 01:00:22.760 you know, every size

NOTE Confidence: 0.559254254

01:00:23.000 --> 01:00:24.400 comes in the same size. Yeah,

NOTE Confidence: 0.458314306666667

01:00:24.560 --> 01:00:25.916 yeah, the arithmetics are always small

NOTE Confidence: 0.572628846666667

01:00:25.920 --> 01:00:27.837 and round and the bags get bigger and bigger

NOTE Confidence: 0.5829912225

01:00:27.840 --> 01:00:29.492 and bigger and bigger. But when when

NOTE Confidence: 0.5829912225

01:00:29.492 --> 01:00:31.360 do you see the immersion? Just like

NOTE Confidence: 0.638526113333333

01:00:31.720 --> 01:00:32.920 self started right

NOTE Confidence: 0.75125650375

01:00:32.920 --> 01:00:34.480 about the same time that they

NOTE Confidence: 0.75125650375

01:00:34.480 --> 01:00:37.451 express the C41. So by the time

NOTE Confidence: 0.75125650375

01:00:37.451 --> 01:00:38.319 they're they're they're 41,

NOTE Confidence: 0.75125650375

01:00:38.320 --> 01:00:39.358 they're they're kind of bigger now.

NOTE Confidence: 0.81822744

01:00:42.240 --> 01:00:43.518 We haven't looked at that carefully.

NOTE Confidence: 0.81822744

01:00:43.520 --> 01:00:45.956 If we really looked at nuclear size

NOTE Confidence: 0.81822744

01:00:45.960 --> 01:00:47.724 carefully, I would bet we would see

NOTE Confidence: 0.81822744

01:00:47.724 --> 01:00:48.880 something different because they're

NOTE Confidence: 0.81822744

01:00:48.880 --> 01:00:50.320 undergoing different nuclear changes.

NOTE Confidence: 0.81822744

01:00:50.320 --> 01:00:51.120 But we haven't looked that

NOTE Confidence: 0.642686613333333

01:00:51.120 --> 01:00:52.278 carefully. Thank you.

NOTE Confidence: 0.4887288

01:00:55.080 --> 01:00:55.840 One last question, the

NOTE Confidence: 0.472317065

01:00:58.080 --> 01:01:01.560 cell regulatory volume is it's super, super

NOTE Confidence: 0.532123136666667

01:01:04.000 --> 01:01:06.034 tightly regulated and that carrier sets

NOTE Confidence: 0.532123136666667

01:01:06.034 --> 01:01:08.080 something very unique in that regard.

NOTE Confidence: 0.532123136666667

01:01:08.080 --> 01:01:10.285 So I was wondering can you stall

NOTE Confidence: 0.532123136666667

01:01:10.285 --> 01:01:13.640 that process and or carrier sets

NOTE Confidence: 0.532123136666667

01:01:13.640 --> 01:01:16.359 that they do not and you know

NOTE Confidence: 0.5947661175

01:01:17.600 --> 01:01:18.640 how long can can

NOTE Confidence: 0.815826193

01:01:18.640 --> 01:01:20.656 that be done or if that

NOTE Confidence: 0.815826193

01:01:20.656 --> 01:01:22.000 happens in any pathologies

NOTE Confidence: 0.827435747777778

01:01:24.560 --> 01:01:27.108 it hasn't been done but many people

NOTE Confidence: 0.827435747777778

01:01:27.108 --> 01:01:29.398 have tried not specifically with cell

NOTE Confidence: 0.827435747777778

01:01:29.398 --> 01:01:31.552 volume because many period sites can

NOTE Confidence: 0.827435747777778

01:01:31.552 --> 01:01:33.400 make platelets as a 2N cell as 4,

NOTE Confidence: 0.827435747777778

01:01:33.400 --> 01:01:36.112 N cell as 8, N as 16 and 32.

NOTE Confidence: 0.827435747777778

01:01:36.112 --> 01:01:37.200 So the question is,

NOTE Confidence: 0.827435747777778

01:01:37.200 --> 01:01:40.182 what tells the men stop undergoing this

NOTE Confidence: 0.827435747777778

01:01:40.182 --> 01:01:43.678 end of mitosis and start making platelets?

NOTE Confidence: 0.827435747777778

01:01:43.680 --> 01:01:46.656 All we know so far is that if you

NOTE Confidence: 0.827435747777778

01:01:46.656 --> 01:01:48.840 take the inside of a magnet making

NOTE Confidence: 0.827435747777778

01:01:48.909 --> 01:01:51.536 platelets set up and Joe Battalion

NOTE Confidence: 0.827435747777778

01:01:51.536 --> 01:01:54.000 and you inject it into a 2NA,

NOTE Confidence: 0.827435747777778

01:01:54.000 --> 01:01:55.626 it'll make platelets.

NOTE Confidence: 0.827435747777778

01:01:55.626 --> 01:01:58.878 So there's something that says go.

NOTE Confidence: 0.827435747777778

01:01:58.880 --> 01:02:00.100 And once you have it,

NOTE Confidence: 0.827435747777778

01:02:00.100 --> 01:02:01.475 you can transplant it into

NOTE Confidence: 0.827435747777778

01:02:01.475 --> 01:02:02.759 another bag and it'll tell

NOTE Confidence: 0.630230093846154

01:02:02.760 --> 01:02:04.776 it to go. So you can give a hypertonic

NOTE Confidence: 0.630230093846154

01:02:04.776 --> 01:02:06.119 shock to a melancharyocyte.

NOTE Confidence: 0.630230093846154

01:02:06.120 --> 01:02:07.960 Would it make platelets? I don't know.

NOTE Confidence: 0.3690446475

01:02:11.280 --> 01:02:12.450 I don't know if that would

NOTE Confidence: 0.3690446475

01:02:12.450 --> 01:02:14.040 be done. I don't know. Thank

NOTE Confidence: 0.757707125

01:02:17.280 --> 01:02:18.200 you. Next.