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

 $00{:}00{:}02{.}132 \dashrightarrow 00{:}00{:}05{.}506$  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

 $00:00:06.920 \longrightarrow 00:00:09.125$  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: 0.8694582466666667

 $00:00:14.920 \rightarrow 00:00:17.360$  and Cell Biology here at Yale. Diane

NOTE Confidence: 0.654115713333333

 $00:00:17.360 \longrightarrow 00:00:20.128$  did her MD PhD training at 10 at

NOTE Confidence: 0.654115713333333

 $00{:}00{:}20{.}128 \dashrightarrow 00{:}00{:}21.826$  Penn and she also followed this

NOTE Confidence: 0.654115713333333

 $00:00:21.826 \rightarrow 00:00:23.318$  by clinical pathology training

NOTE Confidence: 0.928602042

 $00{:}00{:}23.680 \dashrightarrow 00{:}00{:}25.760$  at Penn as well. She

NOTE Confidence: 0.8557028725

 $00{:}00{:}25.760 \dashrightarrow 00{:}00{:}28.665$  moved on to postdoctoral training at Johns

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

NOTE Confidence: 0.8557028725

 $00{:}00{:}31.760 \dashrightarrow 00{:}00{:}34.305$  and since then she's developed an

NOTE Confidence: 0.8557028725

 $00:00:34.305 \longrightarrow 00:00:35.666$  internationally recognized research

NOTE Confidence: 0.8557028725

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

NOTE Confidence: 0.8557028725

 $00:00:38.000 \rightarrow 00:00:40.160$  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

 $00:00:46.093 \rightarrow 00:00:48.502$  characterizing gene products involved

NOTE Confidence: 0.8557028725

 $00{:}00{:}48.502 \dashrightarrow 00{:}00{:}50.878$  in acute megakaryoblastic leukemias,

NOTE Confidence: 0.8557028725

 $00:00:50.880 \longrightarrow 00:00:52.880$  defining transcriptional mechanisms

NOTE Confidence: 0.8557028725

 $00:00:52.880 \rightarrow 00:00:55.880$  that regulate megacaryocyte maturation,

NOTE Confidence: 0.8557028725

 $00{:}00{:}55{.}880 \dashrightarrow 00{:}00{:}57{.}728$  and elucidating factors that regulate

NOTE Confidence: 0.8557028725

 $00:00:57.728 \rightarrow 00:00:59.456$  how the erythroid megacaryocyte

NOTE Confidence: 0.8557028725

 $00:00:59.456 \rightarrow 00:01:01.664$  precursor cell in the bone marrow

NOTE Confidence: 0.8557028725

 $00:01:01.664 \rightarrow 00:01:03.560$  differentiates down the erythroid versus

NOTE Confidence: 0.795772965

 $00:01:03.560 \rightarrow 00:01:05.480$  the platelet lineage.

- NOTE Confidence: 0.795772965
- $00:01:05.480 \longrightarrow 00:01:07.422$  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
- $00{:}01{:}08.280 \dashrightarrow 00{:}01{:}10.576$  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
- $00:01:12.080 \dashrightarrow 00:01:14.120$  director of the Blood Bank, Associate
- NOTE Confidence: 0.892690221428571
- $00:01:14.120 \dashrightarrow 00:01:16.880$  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
- $00{:}01{:}20.640 \dashrightarrow 00{:}01{:}23.594$  And she's also the director of an
- NOTE Confidence: 0.723387105714286
- $00{:}01{:}23.600 \dashrightarrow 00{:}01{:}25.518$  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
- $00{:}01{:}28{.}440 \dashrightarrow 00{:}01{:}30{.}760$  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
- $00:01:32.725 \dashrightarrow 00:01:34.690$  resources for investigators in the
- NOTE Confidence: 0.876656233076923

 $00:01:34.753 \rightarrow 00:01:36.910$  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

 $00:01:39.020 \longrightarrow 00:01:40.560$  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

 $00{:}01{:}42.880 \dashrightarrow 00{:}01{:}44.525$  is a recipient of numerous awards and

NOTE Confidence: 0.841068021666667

 $00{:}01{:}44.525 \dashrightarrow 00{:}01{:}46.370$  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

 $00:01:48.600 \dashrightarrow 00:01:50.399$  the Tibor Greenwald Award from the American

NOTE Confidence: 0.905523254

 $00:01:50.400 \longrightarrow 00:01:52.064$  Association of Blood Banks,

NOTE Confidence: 0.905523254

 $00{:}01{:}52{.}064 \dashrightarrow 00{:}01{:}53{.}880$  and she's been also inducted into

NOTE Confidence: 0.905523254

 $00{:}01{:}53.880 \dashrightarrow 00{:}01{:}55.368$  the National Blood Foundation

NOTE Confidence: 0.905523254

 $00:01:55.368 \longrightarrow 00:01:57.639$  Hall of Fame. There's one local

NOTE Confidence: 0.865799702857143

 $00:01:57.640 \longrightarrow 00:01:59.397$  award that I'd really like to mention.

NOTE Confidence: 0.865799702857143

 $00{:}01{:}59{.}400 \dashrightarrow 00{:}02{:}01{.}560$  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

 $00:02:02.852 \rightarrow 00:02:04.830$  and I think this award really

 $00:02:04.830 \longrightarrow 00:02:06.906$  speaks to her complete dedication

NOTE Confidence: 0.865799702857143

 $00{:}02{:}06{.}906 \dashrightarrow 00{:}02{:}09{.}928$  to advance the success of women

NOTE Confidence: 0.865799702857143

 $00:02:09.928 \rightarrow 00:02:11.216$  and those from underrepresented

NOTE Confidence: 0.865799702857143

 $00:02:11.216 \longrightarrow 00:02:13.426$  groups in science and medicine.

NOTE Confidence: 0.865799702857143

 $00:02:13.426 \rightarrow 00:02:15.880$  She's extremely generous with her time,

NOTE Confidence: 0.865799702857143

 $00:02:15.880 \rightarrow 00:02:18.560$  and despite her many responsibilities,

NOTE Confidence: 0.865799702857143

 $00{:}02{:}18.560 \dashrightarrow 00{:}02{:}20.832$  she always finds time to serve as a

NOTE Confidence: 0.865799702857143

 $00:02:20.832 \dashrightarrow 00:02:22.994$  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

 $00:02:26.880 \longrightarrow 00:02:28.160$  So we are really delighted

NOTE Confidence: 0.865799702857143

 $00:02:28.160 \longrightarrow 00:02:29.552$  that she's taken the time today

NOTE Confidence: 0.865799702857143

 $00{:}02{:}29.552 \dashrightarrow 00{:}02{:}30.800$  to accept her invitation

NOTE Confidence: 0.974645148333333

 $00:02:30.800 \dashrightarrow 00:02:33.480$  and present her work to you. We lcome, Dan.

NOTE Confidence: 0.891239725

 $00:02:38.600 \dashrightarrow 00:02:41.162$  Thanks so much, Karen, for that really

- $00:02:41.162 \rightarrow 00:02:43.079$  nice introduction I should have.
- NOTE Confidence: 0.891239725
- $00{:}02{:}43.080 \dashrightarrow 00{:}02{:}44.074$  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
- $00:02:46.560 \longrightarrow 00:02:47.490$  I really wanted to start
- NOTE Confidence: 0.891239725
- $00{:}02{:}47{.}490 \dashrightarrow 00{:}02{:}48{.}234$  with the title slide.
- NOTE Confidence: 0.891239725
- $00{:}02{:}48{.}240 \dashrightarrow 00{:}02{:}49{.}600$  Because of this beautiful picture.
- NOTE Confidence: 0.891239725
- $00:02:49.600 \rightarrow 00:02:52.237$  I'm going to give too much in this talk.
- NOTE Confidence: 0.891239725
- $00{:}02{:}52{.}240 \dashrightarrow 00{:}02{:}53{.}992$  More than one should put into a one
- NOTE Confidence: 0.891239725
- $00{:}02{:}53{.}992 \dashrightarrow 00{:}02{:}55{.}569$  hour talk because I'm talking to
- NOTE Confidence: 0.891239725
- $00:02:55.569 \dashrightarrow 00:02:57.201$  pathology and I just couldn't not
- NOTE Confidence: 0.891239725
- $00{:}02{:}57{.}257 \dashrightarrow 00{:}02{:}58{.}969$  present some of the stuff in our lab
- NOTE Confidence: 0.891239725
- $00:02:58.969 \rightarrow 00:03:00.936$  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
- $00:03:03.240 \rightarrow 00:03:05.260$  pathology trainees and faculty to
- NOTE Confidence: 0.891239725
- $00:03:05.260 \rightarrow 00:03:07.959$  collaborate on some of the the work.
- NOTE Confidence: 0.891239725
- $00:03:07.960 \longrightarrow 00:03:09.568$  But I'll tell you mostly what's

- NOTE Confidence: 0.891239725
- $00{:}03{:}09{.}568 \dashrightarrow 00{:}03{:}10{.}640$  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
- $00:03:14.440 \longrightarrow 00:03:16.414$  And what you can see is that
- NOTE Confidence: 0.891239725
- $00{:}03{:}16{.}414 \dashrightarrow 00{:}03{:}18{.}000$  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
- $00:03:20.360 \rightarrow 00:03:23.118$  the Golgi and the endoplasmic reticulum.
- NOTE Confidence: 0.891239725
- $00{:}03{:}23.120 \dashrightarrow 00{:}03{:}25.395$  And what this is, is expansion microscopy.
- NOTE Confidence: 0.891239725
- $00:03:25.400 \longrightarrow 00:03:27.157$  So this was taken with the confocal,
- NOTE Confidence: 0.891239725
- $00:03:27.160 \longrightarrow 00:03:28.525$  but you really have a lot of
- NOTE Confidence: 0.891239725
- $00:03:28.525 \longrightarrow 00:03:30.360$  the kind of detail that you can
- NOTE Confidence: 0.891239725
- $00:03:30.360 \dashrightarrow 00:03:31.556$  get with electron microscopy.
- NOTE Confidence: 0.891239725
- $00{:}03{:}31{.}560 \dashrightarrow 00{:}03{:}33{.}240$  So it's a pretty picture,
- NOTE Confidence: 0.891239725
- $00:03:33.240 \dashrightarrow 00:03:34.815$  but what I'll be telling you about
- NOTE Confidence: 0.891239725
- $00{:}03{:}34{.}815 \dashrightarrow 00{:}03{:}35{.}923$  today is hematopoies is For those
- NOTE Confidence: 0.891239725

 $00:03:35.923 \rightarrow 00:03:37.274$  of you who don't think about this,

NOTE Confidence: 0.891239725

 $00:03:37.280 \longrightarrow 00:03:39.072$  it in our bone marrow there's a

NOTE Confidence: 0.891239725

 $00:03:39.072 \dashrightarrow 00:03:40.347$ 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

 $00{:}03{:}41{.}943 \dashrightarrow 00{:}03{:}43{.}980$  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

 $00{:}03{:}44.859 \dashrightarrow 00{:}03{:}46.396$  stem cell differentiates into all

NOTE Confidence: 0.891239725

 $00:03:46.396 \dashrightarrow 00:03:48.580$  of the cells in our peripheral blood

NOTE Confidence: 0.891239725

 $00{:}03{:}48.580 \dashrightarrow 00{:}03{:}50.200$  leukocytes as well as the red,

NOTE Confidence: 0.891239725

 $00:03:50.200 \dashrightarrow 00:03:52.720$  red blood cells and platelets.

NOTE Confidence: 0.891239725

 $00{:}03{:}52{.}720 \dashrightarrow 00{:}03{:}55{.}261$  And my lab really focuses on this

NOTE Confidence: 0.891239725

 $00:03:55.261 \rightarrow 00:03:58.235$  bright orange cell which we have here as MEP.

NOTE Confidence: 0.891239725

 $00:03:58.240 \longrightarrow 00:04:00.400$  I'm going to try not to talk in

NOTE Confidence: 0.891239725

 $00:04:00.400 \longrightarrow 00:04:01.960$  too many abbreviations,

NOTE Confidence: 0.891239725

 $00{:}04{:}01{.}960 \dashrightarrow 00{:}04{:}04{.}624$  but the name of the MEP is a

NOTE Confidence: 0.891239725

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

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

NOTE Confidence: 0.891239725

 $00:04:07.760 \rightarrow 00:04:09.960$  so I'll only sometimes say the whole thing.

NOTE Confidence: 0.891239725

 $00:04:09.960 \rightarrow 00:04:12.221$  So this is the bipotent precursor of

NOTE Confidence: 0.891239725

 $00{:}04{:}12{.}221 \dashrightarrow 00{:}04{:}13{.}957$  megacary cytes that make platelets and

NOTE Confidence: 0.891239725

 $00:04:13.957 \longrightarrow 00:04:16.087$  the erythroid lineage that ends up

NOTE Confidence: 0.891239725

 $00:04:16.087 \dashrightarrow 00:04:17.719$  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

 $00:04:21.120 \longrightarrow 00:04:22.626$  we make about 2,000,000 platelets and

NOTE Confidence: 0.96920333

 $00:04:22.626 \rightarrow 00:04:24.518$  2 million red blood cells every second.

NOTE Confidence: 0.96920333

 $00:04:24.520 \longrightarrow 00:04:27.364$  So this cell is very busy making its

NOTE Confidence: 0.96920333

 $00{:}04{:}27.364 \dashrightarrow 00{:}04{:}29.474$  progenitors and trying to decide.

NOTE Confidence: 0.96920333

 $00{:}04{:}29{.}480 \dashrightarrow 00{:}04{:}31{.}316$  I I don't really love using the word decide,

NOTE Confidence: 0.96920333

 $00:04:31.320 \longrightarrow 00:04:33.609$  but it really helps you ask the

NOTE Confidence: 0.96920333

 $00{:}04{:}33{.}609 \dashrightarrow 00{:}04{:}35{.}319$  question which lineage to go down.

NOTE Confidence: 0.96920333

 $00{:}04{:}35{.}320 \dashrightarrow 00{:}04{:}38{.}230$  So what is determining the fate

 $00:04:38.230 \rightarrow 00:04:41.120$  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

 $00:04:42.450 \longrightarrow 00:04:43.514$  complete without saying this,

NOTE Confidence: 0.96920333

 $00{:}04{:}43.520 \dashrightarrow 00{:}04{:}45.494$  there is evidence in the literature that

NOTE Confidence: 0.96920333

 $00{:}04{:}45{.}494 \dashrightarrow 00{:}04{:}47{.}672$  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

 $00{:}04{:}49{.}600 \dashrightarrow 00{:}04{:}51{.}796$  So if that is the case,

NOTE Confidence: 0.96920333

 $00:04:51.800 \rightarrow 00:04:53.851$  then I'm not talking about that lineage

NOTE Confidence: 0.96920333

 $00{:}04{:}53.851 \dashrightarrow 00{:}04{:}55.760$  to megacary cytes, I'm talking about

NOTE Confidence: 0.96920333

 $00{:}04{:}55.760 \dashrightarrow 00{:}04{:}57.960$  this bipotent lineage to megacary cytes.

NOTE Confidence: 0.96920333

 $00:04:57.960 \longrightarrow 00:04:59.400$  Why did we pick MEP?

NOTE Confidence: 0.96920333

 $00:04:59.400 \longrightarrow 00:05:00.416$  Well, first of all,

NOTE Confidence: 0.96920333

 $00{:}05{:}00{.}416 \dashrightarrow 00{:}05{:}02{.}286$  it's a model of bipotent fate specification

NOTE Confidence: 0.96920333

 $00:05:02.286 \longrightarrow 00:05:04.288$  which is important in all of the

NOTE Confidence: 0.96920333

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

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 $00:05:06.160 \rightarrow 00:05:09.520$  tissue repair and response to injury.

 $00:05:09.520 \longrightarrow 00:05:09.933$  Secondly,

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 $00{:}05{:}09{.}933 \dashrightarrow 00{:}05{:}11{.}998$  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

 $00:05:13.880 \longrightarrow 00:05:15.576$  the place that we get our red cells

NOTE Confidence: 0.96920333

 $00{:}05{:}15{.}576 \dashrightarrow 00{:}05{:}17{.}051$  and platelets that we transfuse into

NOTE Confidence: 0.96920333

 $00{:}05{:}17.051 \dashrightarrow 00{:}05{:}18.557$  patients is from healthy donors and

NOTE Confidence: 0.96920333

 $00:05:18.603 \dashrightarrow 00:05:20.115$  there really aren't enough of them.

NOTE Confidence: 0.96920333

 $00{:}05{:}20{.}120 \dashrightarrow 00{:}05{:}22{.}199$  And there's a huge amount of work

NOTE Confidence: 0.96920333

 $00{:}05{:}22.199 \dashrightarrow 00{:}05{:}24.277$  in finding and collecting cells from

NOTE Confidence: 0.96920333

 $00{:}05{:}24.277 \dashrightarrow 00{:}05{:}26.881$  healthy donors in order to maintain an

NOTE Confidence: 0.96920333

 $00{:}05{:}26{.}948 \dashrightarrow 00{:}05{:}29{.}118$  adequate supply for the recipients.

NOTE Confidence: 0.96920333

 $00{:}05{:}29{.}120 \dashrightarrow 00{:}05{:}30{.}554$  And sometimes we really run low

NOTE Confidence: 0.96920333

 $00:05:30.554 \rightarrow 00:05:32.120$  on platelets in red blood cells,

NOTE Confidence: 0.96920333

 $00{:}05{:}32.120 \dashrightarrow 00{:}05{:}34.640$  particularly in the last year or so.

NOTE Confidence: 0.96920333

 $00{:}05{:}34{.}640 \dashrightarrow 00{:}05{:}36{.}344$  We've had several times when we're

- $00:05:36.344 \rightarrow 00:05:37.196$  near crisis situation.
- NOTE Confidence: 0.96920333
- $00{:}05{:}37{.}200 \dashrightarrow 00{:}05{:}38{.}676$  So if we could figure out a way to
- NOTE Confidence: 0.96920333
- $00:05:38.676 \dashrightarrow 00:05:40.600$  make them in vitro, that would be great.
- NOTE Confidence: 0.96920333
- $00:05:40.600 \rightarrow 00:05:41.160$  And finally,
- NOTE Confidence: 0.96920333
- $00:05:41.160 \longrightarrow 00:05:43.200$  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
- $00{:}05{:}51{.}936 \dashrightarrow 00{:}05{:}54{.}240$  What you have to do is a colony
- NOTE Confidence: 0.96920333
- $00:05:54.304 \longrightarrow 00:05:55.200$  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
- $00:05:56.680 \rightarrow 00:05:57.760$  it's going to form a colony.
- NOTE Confidence: 0.96920333
- $00:05:57.760 \rightarrow 00:06:00.000$  When we do him out of aquatic assays,
- NOTE Confidence: 0.96920333
- $00:06:00.000 \rightarrow 00:06:01.638$  we take a stemmer progenitor cell,
- NOTE Confidence: 0.96920333
- $00:06:01.640 \longrightarrow 00:06:03.328$  we put it into a semi solid medium

 $00:06:03.328 \rightarrow 00:06:05.891$  in a very dilute fashion and if that

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 $00:06:05.891 \rightarrow 00:06:07.279$  cell divides and differentiates,

NOTE Confidence: 0.96920333

 $00:06:07.280 \longrightarrow 00:06:09.800$  it's going to form a colony of cells.

NOTE Confidence: 0.96920333

 $00:06:09.800 \dashrightarrow 00:06:11.609$  And what we do is we over the course

NOTE Confidence: 0.96920333

 $00{:}06{:}11.609 \dashrightarrow 00{:}06{:}13.571$  of the two weeks that cell makes 2

NOTE Confidence: 0.96920333

 $00:06:13.571 \rightarrow 00:06:15.490$  cell types with one of them being

NOTE Confidence: 0.96920333

 $00:06:15.490 \dashrightarrow 00:06:16.910$  megakaryocytes and other cells of

NOTE Confidence: 0.96920333

 $00:06:16.910 \longrightarrow 00:06:17.896$  the erythroid lineage.

NOTE Confidence: 0.96920333

 $00{:}06{:}17.896 \dashrightarrow 00{:}06{:}19.984$  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

 $00:06:21.200 \longrightarrow 00:06:24.360$  the bipotent progenitor that is

NOTE Confidence: 0.96920333

 $00{:}06{:}24.360 \dashrightarrow 00{:}06{:}25.480$  the assay we used.

NOTE Confidence: 0.96920333

 $00{:}06{:}25{.}480 \dashrightarrow 00{:}06{:}27{.}952$  We identified in this paper

NOTE Confidence: 0.96920333

 $00:06:27.952 \longrightarrow 00:06:30.751$  from 2016 a really good sorting

NOTE Confidence: 0.96920333

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

 $00{:}06{:}33{.}400 \dashrightarrow 00{:}06{:}35{.}872$  What we did is we worked out the

NOTE Confidence: 0.96920333

 $00{:}06{:}35{.}872 \dashrightarrow 00{:}06{:}38{.}576$  as say 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

 $00{:}06{:}40{.}510 \dashrightarrow 00{:}06{:}43{.}120$  the best possible way of isolating the cells.

NOTE Confidence: 0.96920333

 $00{:}06{:}43.120 \dashrightarrow 00{:}06{:}44.904$  What happens is after the course of two

NOTE Confidence: 0.96920333

 $00:06:44.904 \rightarrow 00:06:46.678$  weeks a single cell forms a colony.

NOTE Confidence: 0.96920333

 $00:06:46.680 \longrightarrow 00:06:48.198$  This is a colony of cells.

NOTE Confidence: 0.96920333

 $00:06:48.200 \longrightarrow 00:06:49.892$  It's been stained with anti glycoporin

NOTE Confidence: 0.96920333

 $00{:}06{:}49{.}892 \dashrightarrow 00{:}06{:}52{.}070$  A which is a surface marker for

NOTE Confidence: 0.96920333

 $00:06:52.070 \longrightarrow 00:06:53.394$  red blood cell lineage.

NOTE Confidence: 0.96920333

 $00{:}06{:}53{.}400 \dashrightarrow 00{:}06{:}55{.}104$  This and this colony is entirely

NOTE Confidence: 0.96920333

 $00{:}06{:}55{.}104 \dashrightarrow 00{:}06{:}56{.}988$  made-up of cells that are committed

NOTE Confidence: 0.96920333

 $00:06:56.988 \longrightarrow 00:06:58.360$  to the erythroid lineage.

NOTE Confidence: 0.96920333

 $00{:}06{:}58{.}360 \dashrightarrow 00{:}07{:}00{.}200$  Here's a colony that's stained

NOTE Confidence: 0.96920333

 $00:07:00.200 \longrightarrow 00:07:02.040$  with anti CD 41 only

NOTE Confidence: 0.74838563444445

 $00:07:02.040 \dashrightarrow 00:07:04.119$  CD 41 is on the mega carry site lineage.

 $00:07:04.120 \dashrightarrow 00:07:06.127$  So this is a colony of cells that are

NOTE Confidence: 0.74838563444445

 $00:07:06.127 \rightarrow 00:07:08.303$  mega carry site only and then we often

NOTE Confidence: 0.74838563444445

 $00{:}07{:}08.303 \dashrightarrow 00{:}07{:}10.841$  get colonies that have cells of both the

NOTE Confidence: 0.74838563444445

 $00:07:10.841 \rightarrow 00:07:12.511$  megakaryocyte and the erythroid lineage.

NOTE Confidence: 0.74838563444445

 $00:07:12.520 \longrightarrow 00:07:13.600$  And just to be more complete,

NOTE Confidence: 0.74838563444445

 $00{:}07{:}13.600 \dashrightarrow 00{:}07{:}15.841$  my lab has now switched to an assay and

NOTE Confidence: 0.74838563444445

 $00:07:15.841 \rightarrow 00:07:17.369$  rather than using immunohistochemistry for

NOTE Confidence: 0.74838563444445

 $00:07:17.369 \rightarrow 00:07:20.280$  glia in 41 we now do immunofluorescence.

NOTE Confidence: 0.878716792666667

 $00{:}07{:}22.840 \dashrightarrow 00{:}07{:}24.618$  Based on the data obtained we now

NOTE Confidence: 0.878716792666667

 $00:07:24.618 \rightarrow 00:07:27.223$  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

 $00:07:28.999 \longrightarrow 00:07:31.191$  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

 $00{:}07{:}33.745 \dashrightarrow 00{:}07{:}36.355$  and of those colonies about 50%

NOTE Confidence: 0.878716792666667

 $00:07:36.360 \longrightarrow 00:07:39.066$  shown here in blue are cells are

 $00{:}07{:}39.066 \dashrightarrow 00{:}07{:}40.952$  comprised of cells with both of cells

NOTE Confidence: 0.878716792666667

 $00{:}07{:}40.952 \dashrightarrow 00{:}07{:}42.884$  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

 $00:07:44.500 \rightarrow 00:07:46.432$  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: 0.8787167926666667

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

NOTE Confidence: 0.878716792666667

 $00:07:49.800 \rightarrow 00:07:51.560$  with most of the colonies are mega only NOTE Confidence: 0.878716792666667

 $00{:}07{:}51{.}560 \dashrightarrow 00{:}07{:}53{.}573$  and the erythroid progenitis under your

NOTE Confidence: 0.878716792666667

 $00:07:53.573 \rightarrow 00:07:55.473$  similarly where they're mostly erythroid.

NOTE Confidence: 0.878716792666667

 $00:07:55.480 \longrightarrow 00:07:56.992$  One of the questions you may ask you NOTE Confidence: 0.8787167926666667

 $00{:}07{:}56{.}992 \dashrightarrow 00{:}07{:}58{.}722$  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

 $00{:}08{:}00{.}251 \dashrightarrow 00{:}08{:}01{.}880$  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

 $00{:}08{:}04.000 \dashrightarrow 00{:}08{:}05.400$  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.

 $00:08:06.760 \longrightarrow 00:08:08.832$  And what I'm going to tell you is

NOTE Confidence: 0.878716792666667

 $00:08:08.832 \longrightarrow 00:08:10.723$  that the data very strongly suggest

NOTE Confidence: 0.878716792666667

 $00{:}08{:}10.723 \dashrightarrow 00{:}08{:}13.450$  that what we have is quite a pure

NOTE Confidence: 0.878716792666667

 $00:08:13.450 \longrightarrow 00:08:15.460$  population and that there is a

NOTE Confidence: 0.878716792666667

 $00{:}08{:}15{.}460 \dashrightarrow 00{:}08{:}17{.}120$  probability that a bipotent cell,

NOTE Confidence: 0.878716792666667

 $00:08:17.120 \longrightarrow 00:08:19.478$  when put into the culture will,

NOTE Confidence: 0.878716792666667

 $00:08:19.480 \longrightarrow 00:08:20.576$  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

 $00:08:22.640 \longrightarrow 00:08:24.112$  subsequently all decide Erythroid

NOTE Confidence: 0.878716792666667

 $00:08:24.112 \rightarrow 00:08:25.952$  or subsequently all decide Meg.

NOTE Confidence: 0.878716792666667

 $00{:}08{:}25{.}960 \dashrightarrow 00{:}08{:}27{.}647$  And it doesn't mean that the starting

NOTE Confidence: 0.878716792666667

 $00{:}08{:}27.647 \dashrightarrow 00{:}08{:}28.915$  cell didn't have the potential

NOTE Confidence: 0.878716792666667

 $00{:}08{:}28{.}915 \dashrightarrow 00{:}08{:}30{.}160$  to go down both lineages,

NOTE Confidence: 0.878716792666667

 $00{:}08{:}30{.}160 \dashrightarrow 00{:}08{:}33{.}280$  and I'll try to convince you of that.

NOTE Confidence: 0.878716792666667

 $00{:}08{:}33{.}280 \dashrightarrow 00{:}08{:}34{.}972$  So this enrichment of these populations

 $00:08:34.972 \longrightarrow 00:08:37.073$  has allowed us to study the fate

NOTE Confidence: 0.878716792666667

 $00{:}08{:}37{.}073 \dashrightarrow 00{:}08{:}38{.}301$  transitions from the bipotent

NOTE Confidence: 0.878716792666667

 $00:08:38.301 \rightarrow 00:08:39.903$  progenitor to the Meg progenitor

NOTE Confidence: 0.878716792666667

 $00:08:39.903 \rightarrow 00:08:41.483$  and from the bipotent progenitor

NOTE Confidence: 0.878716792666667

 $00:08:41.483 \longrightarrow 00:08:44.520$  to the erythroid progenitor.

NOTE Confidence: 0.878716792666667

 $00:08:44.520 \rightarrow 00:08:46.812$  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

 $00:08:48.360 \longrightarrow 00:08:49.395$  but quickly enough that I'm

NOTE Confidence: 0.878716792666667

 $00{:}08{:}49{.}395 \dashrightarrow 00{:}08{:}50{.}879$  done by the end of the hour.

NOTE Confidence: 0.878716792666667

 $00:08:50.880 \longrightarrow 00:08:53.071$  The 1st is some really novel data

NOTE Confidence: 0.878716792666667

 $00{:}08{:}53{.}071 \dashrightarrow 00{:}08{:}56{.}045$  that came out of our single cell RNA

NOTE Confidence: 0.878716792666667

 $00:08:56.045 \rightarrow 00:08:58.010$  sequencing of these populations that

NOTE Confidence: 0.878716792666667

 $00{:}08{:}58{.}080 \dashrightarrow 00{:}09{:}00{.}240$  revealed that the cell cycle speed

NOTE Confidence: 0.878716792666667

 $00:09:00.240 \longrightarrow 00:09:02.916$  of the MEP actually seems to predict

NOTE Confidence: 0.878716792666667

 $00:09:02.916 \rightarrow 00:09:05.388$  whether that's going to be megacaryocyte

NOTE Confidence: 0.878716792666667

 $00:09:05.388 \rightarrow 00:09:07.995$  output or erythroid output and that we

 $00:09:07.995 \longrightarrow 00:09:10.426$  can actually toggle the fate of the

NOTE Confidence: 0.878716792666667

 $00:09:10.426 \rightarrow 00:09:12.631$  MEP by toggling its cell cycle speed.

NOTE Confidence: 0.878716792666667

 $00:09:12.640 \longrightarrow 00:09:14.464$  Then I'll tell you about the role of

NOTE Confidence: 0.878716792666667

 $00{:}09{:}14.464 \dashrightarrow 00{:}09{:}16.460$  the Runks 1 transcription factor and

NOTE Confidence: 0.878716792666667

 $00{:}09{:}16.460 \dashrightarrow 00{:}09{:}18.320$  how it's post translational modification

NOTE Confidence: 0.878716792666667

 $00:09:18.320 \dashrightarrow 00:09:20.479$  effects MEP fate and then we'll talk about,

NOTE Confidence: 0.878716792666667

 $00:09:20.480 \longrightarrow 00:09:22.405$  we'll show show you some really cool

NOTE Confidence: 0.878716792666667

 $00{:}09{:}22.405 \dashrightarrow 00{:}09{:}24.275$  data watching MEP fate specification that

NOTE Confidence: 0.878716792666667

 $00:09:24.275 \longrightarrow 00:09:26.261$  really gave us those probabilities that

NOTE Confidence: 0.878716792666667

 $00:09:26.261 \longrightarrow 00:09:28.318$  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

 $00{:}09{:}32.160 \dashrightarrow 00{:}09{:}33.400$  that I already introduced

NOTE Confidence: 0.73381781444444

 $00{:}09{:}36{.}720 \dashrightarrow 00{:}09{:}38{.}997$  what we did once we had fact sort facts,

NOTE Confidence: 0.73381781444444

 $00:09:39.000 \rightarrow 00:09:42.040$  gating strategies for enriching MEP,

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

NOTE Confidence: 0.73381781444444

 $00{:}09{:}44.280 \dashrightarrow 00{:}09{:}46.170$  We also sorted the upstream common

NOTE Confidence: 0.73381781444444

 $00{:}09{:}46{.}170 \dashrightarrow 00{:}09{:}48{.}053$  myeloid progenitors and we sent these

NOTE Confidence: 0.73381781444444

 $00:09:48.053 \rightarrow 00:09:49.757$  for single cell RNA SEC analysis.

NOTE Confidence: 0.73381781444444

 $00{:}09{:}49{.}760 \dashrightarrow 00{:}09{:}51{.}112$  And this was work done by Yi Shan

NOTE Confidence: 0.73381781444444

 $00:09:51.112 \longrightarrow 00:09:53.344$  Liu in the lab, an amazing post doc NOTE Confidence: 0.73381781444444

 $00:09:53.344 \longrightarrow 00:09:55.600$  who published this work in 2018.

NOTE Confidence: 0.73381781444444

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

NOTE Confidence: 0.73381781444444

 $00{:}09{:}57{.}035 \dashrightarrow 00{:}09{:}58{.}781$  at the single cell RNA SEC and if

NOTE Confidence: 0.73381781444444

 $00:09:58.781 \rightarrow 00:10:00.280$  you're not used to looking at this,

NOTE Confidence: 0.73381781444444

 $00{:}10{:}00{.}280 \dashrightarrow 00{:}10{:}03{.}577$  the data from the individual cells has

NOTE Confidence: 0.73381781444444

 $00{:}10{:}03.577 \dashrightarrow 00{:}10{:}06.652$  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: 0.73381781444444

 $00:10:09.560 \longrightarrow 00:10:11.600$  the MEP or the Meg erythroid

NOTE Confidence: 0.733817814444444

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

NOTE Confidence: 0.73381781444444

 $00:10:12.280 \longrightarrow 00:10:14.116$  the Meg progenitors or the Meg

- NOTE Confidence: 0.73381781444444
- $00:10:14.116 \longrightarrow 00:10:15.340$  committed and the erythroid
- NOTE Confidence: 0.73381781444444
- $00:10:15.393 \rightarrow 00:10:17.237$  progenitors or Erythroid committed.
- NOTE Confidence: 0.73381781444444
- $00:10:17.240 \longrightarrow 00:10:18.591$  And what you can see is when
- NOTE Confidence: 0.73381781444444
- $00:10:18.591 \longrightarrow 00:10:19.880$  we fact sort out these MEP,
- NOTE Confidence: 0.73381781444444
- 00:10:19.880 --> 00:10:22.200 it's really a distinct population.
- NOTE Confidence: 0.73381781444444
- $00{:}10{:}22.200 \dashrightarrow 00{:}10{:}23.680$  There's a bit of a graduation to it,
- NOTE Confidence: 0.73381781444444
- $00:10:23.680 \longrightarrow 00:10:25.320$  but it's a distinct population.
- NOTE Confidence: 0.73381781444444
- 00:10:25.320 --> 00:10:28.395 It looks very different from CMPMKP or ERP,
- NOTE Confidence: 0.73381781444444
- $00{:}10{:}28{.}395 \dashrightarrow 00{:}10{:}30{.}252$  but it looks like it had still has
- NOTE Confidence: 0.73381781444444
- $00{:}10{:}30.252 \dashrightarrow 00{:}10{:}31.827$  some genes that are still on from
- NOTE Confidence: 0.73381781444444
- $00:10:31.827 \rightarrow 00:10:33.713$  the CMP that are going to be turned
- NOTE Confidence: 0.73381781444444
- $00{:}10{:}33{.}713 \dashrightarrow 00{:}10{:}36{.}292$  off and some genes that are on in
- NOTE Confidence: 0.73381781444444
- $00:10:36.292 \rightarrow 00:10:38.714$  erythroid and mega caries like Destin
- NOTE Confidence: 0.73381781444444
- $00{:}10{:}38{.}714 \dashrightarrow 00{:}10{:}40{.}838$  cells that are just coming on.
- NOTE Confidence: 0.73381781444444
- $00:10:40.840 \rightarrow 00:10:44.319$  So it really is a transitional state.
- NOTE Confidence: 0.73381781444444

 $00:10:44.320 \longrightarrow 00:10:46.770$  When we looked at the gene expression

NOTE Confidence: 0.73381781444444

 $00{:}10{:}46.770 \dashrightarrow 00{:}10{:}48.912$  analysis and compared MEP to the

NOTE Confidence: 0.73381781444444

 $00:10:48.912 \longrightarrow 00:10:49.600$  other populations,

NOTE Confidence: 0.73381781444444

 $00:10:49.600 \rightarrow 00:10:51.850$  what we found that the pathways

NOTE Confidence: 0.73381781444444

 $00{:}10{:}51{.}850 \dashrightarrow 00{:}10{:}54{.}259$  that were over represented in the

NOTE Confidence: 0.73381781444444

 $00{:}10{:}54.259 \dashrightarrow 00{:}10{:}55.519$  differentially expressed genes

NOTE Confidence: 0.73381781444444

 $00:10:55.519 \rightarrow 00:10:58.080$  were almost always the cell cycle.

NOTE Confidence: 0.73381781444444

 $00:10:58.080 \longrightarrow 00:10:59.744$  And so you can see it's here from

NOTE Confidence: 0.73381781444444

 $00{:}10{:}59{.}744 \dashrightarrow 00{:}11{:}01{.}555$  the MEP to the Meg progenitor cell

NOTE Confidence: 0.73381781444444

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

NOTE Confidence: 0.73381781444444

 $00{:}11{:}02{.}360 \dashrightarrow 00{:}11{:}04{.}320$  from the MEP to the erythroid shows up

NOTE Confidence: 0.73381781444444

 $00:11:04.320 \rightarrow 00:11:06.835$  and the other things were were less specific.

NOTE Confidence: 0.73381781444444

 $00:11:06.840 \dashrightarrow 00:11:08.575$  We weren't entirely surprised by

NOTE Confidence: 0.73381781444444

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

NOTE Confidence: 0.73381781444444

 $00{:}11{:}10.367 \dashrightarrow 00{:}11{:}12.317$  data that were consistent with this.

NOTE Confidence: 0.73381781444444

 $00:11:12.320 \rightarrow 00:11:13.958$  What we had done prior to getting

 $00{:}11{:}13.958 \dashrightarrow 00{:}11{:}16.067$  the single cell RNA C data is we had

NOTE Confidence: 0.73381781444444

 $00:11:16.067 \rightarrow 00:11:17.273$  tried a candidate approach where

NOTE Confidence: 0.73381781444444

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

NOTE Confidence: 0.73381781444444

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

NOTE Confidence: 0.73381781444444

 $00:11:21.600 \rightarrow 00:11:23.232$  affected their hematopoietic output.

NOTE Confidence: 0.73381781444444

 $00:11:23.240 \longrightarrow 00:11:25.144$  We already knew that in response to

NOTE Confidence: 0.73381781444444

 $00:11:25.144 \rightarrow 00:11:26.799$  all trans retinoic acid which goes

NOTE Confidence: 0.73381781444444

 $00:11:26.799 \rightarrow 00:11:28.633$  to the nucleus and binds directly as

NOTE Confidence: 0.73381781444444

 $00{:}11{:}28.686 \dashrightarrow 00{:}11{:}30.396$  a transcription factor on the DNA,

NOTE Confidence: 0.73381781444444

 $00{:}11{:}30{.}400 \dashrightarrow 00{:}11{:}33{.}214$  that we had a dose dependent increase

NOTE Confidence: 0.73381781444444

 $00:11:33.214 \rightarrow 00:11:35.253$  in megacaryocyte only colonies when

NOTE Confidence: 0.73381781444444

 $00{:}11{:}35{.}253 \dashrightarrow 00{:}11{:}36{.}396$  we added ATRA.

NOTE Confidence: 0.73381781444444

 $00{:}11{:}36{.}400 \dashrightarrow 00{:}11{:}38{.}003$  We also knew that when we added

NOTE Confidence: 0.73381781444444

 $00{:}11{:}38{.}003 \dashrightarrow 00{:}11{:}39{.}558$  rapamyc in which is an mtor inhibitor,

NOTE Confidence: 0.73381781444444

 $00{:}11{:}39.560 \dashrightarrow 00{:}11{:}40.622$  it's affecting metabolism.

 $00:11:40.622 \rightarrow 00:11:43.601$  We had a similarly A dose dependent

NOTE Confidence: 0.73381781444444

 $00:11:43.601 \rightarrow 00:11:45.766$  increase in megacaryocyte biased and

NOTE Confidence: 0.73381781444444

 $00{:}11{:}45.766 \dashrightarrow 00{:}11{:}48.604$  what we realized is that both ATRA

NOTE Confidence: 0.73381781444444

 $00:11:48.604 \rightarrow 00:11:51.033$  and rapamycin can slow the cell cycle.

NOTE Confidence: 0.73381781444444

 $00{:}11{:}51{.}040 \dashrightarrow 00{:}11{:}52{.}784$  So we tested that.

NOTE Confidence: 0.73381781444444

 $00:11:52.784 \rightarrow 00:11:56.632$  What we've done here is a dilution assay,

NOTE Confidence: 0.73381781444444

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

NOTE Confidence: 0.73381781444444

 $00:11:58.480 \longrightarrow 00:11:59.628$  for those of you who are not

NOTE Confidence: 0.73381781444444

 $00:11:59.628 \longrightarrow 00:12:00.120$  familiar with this,

NOTE Confidence: 0.73381781444444

 $00:12:00.120 \longrightarrow 00:12:01.835$  you stain all your cells at time

NOTE Confidence: 0.73381781444444

 $00{:}12{:}01.835 \dashrightarrow 00{:}12{:}03.520$  zero with a fluorescent dye.

NOTE Confidence: 0.73381781444444

 $00:12:03.520 \rightarrow 00:12:05.080$  Each time the cells divide,

NOTE Confidence: 0.733817814444444

 $00{:}12{:}05{.}080 \dashrightarrow 00{:}12{:}06{.}837$  they have less of the fluorescent dye.

NOTE Confidence: 0.73381781444444

 $00:12:06.840 \longrightarrow 00:12:08.760$  So the further to the left they are,

NOTE Confidence: 0.73381781444444

 $00:12:08.760 \longrightarrow 00:12:10.720$  the more division there's been.

NOTE Confidence: 0.73381781444444

 $00:12:10.720 \longrightarrow 00:12:12.253$  And what you can see is the

- NOTE Confidence: 0.73381781444444
- $00:12:12.253 \longrightarrow 00:12:13.640$  controls here are shown in blue.
- NOTE Confidence: 0.73381781444444
- 00:12:13.640 --> 00:12:14.680 When you treat with ATRA,
- NOTE Confidence: 0.924257633333333
- $00:12:14.680 \rightarrow 00:12:16.136$  there's less division. Similarly,
- NOTE Confidence: 0.924257633333333
- $00:12:16.136 \longrightarrow 00:12:17.956$  when you treat with rapamycin,
- NOTE Confidence: 0.924257633333333
- $00:12:17.960 \rightarrow 00:12:20.018$  there's been less division proving that
- NOTE Confidence: 0.924257633333333
- $00:12:20.018 \rightarrow 00:12:22.039$  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
- $00:12:22.905 \rightarrow 00:12:24.159$  about the speed of the cell cycle,
- NOTE Confidence: 0.924257633333333
- $00:12:24.160 \longrightarrow 00:12:25.918$  we haven't tested that, but there's
- NOTE Confidence: 0.924257633333333
- $00:12:25.920 \rightarrow 00:12:27.640$  they're dividing less frequently.
- NOTE Confidence: 0.941363015833333
- $00{:}12{:}29.680 \dashrightarrow 00{:}12{:}31.129$  What we did next then is just
- NOTE Confidence: 0.941363015833333
- $00:12:31.129 \longrightarrow 00:12:32.639$  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
- $00{:}12{:}34{.}320 \dashrightarrow 00{:}12{:}36{.}640$  These cells completely stopped dividing.
- NOTE Confidence: 0.941363015833333
- $00{:}12{:}36{.}640 \dashrightarrow 00{:}12{:}39{.}232$  We then washed that out and put them
- NOTE Confidence: 0.941363015833333

 $00:12:39.232 \rightarrow 00:12:42.072$  into the colony assays and again saw

NOTE Confidence: 0.941363015833333

 $00{:}12{:}42.072 \dashrightarrow 00{:}12{:}44.704$  this dose dependent increase in the

NOTE Confidence: 0.941363015833333

 $00{:}12{:}44.704 \dashrightarrow 00{:}12{:}46.864$  mega carrier site lineage specification

NOTE Confidence: 0.941363015833333

 $00:12:46.864 \rightarrow 00:12:49.080$  of the MEP that proved this long.

NOTE Confidence: 0.941363015833333

 $00:12:49.080 \longrightarrow 00:12:50.960$  The cell cycle gave us a Meg bias.

NOTE Confidence: 0.941363015833333

 $00:12:50.960 \longrightarrow 00:12:52.136$  But what happens if you speed

NOTE Confidence: 0.941363015833333

 $00:12:52.136 \longrightarrow 00:12:52.920$  up the cell cycle?

NOTE Confidence: 0.941363015833333

 $00:12:52.920 \rightarrow 00:12:54.315$  Well how do you speed up the cell cycle?

NOTE Confidence: 0.941363015833333

 $00{:}12{:}54{.}320 \dashrightarrow 00{:}12{:}56{.}744$  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

 $00{:}12{:}58{.}288 \dashrightarrow 00{:}13{:}00{.}912$  The CDK is that was pretty much toxic

NOTE Confidence: 0.941363015833333

 $00{:}13{:}00{.}912 \dashrightarrow 00{:}13{:}03{.}196$  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

 $00:13:04.761 \rightarrow 00:13:06.679$  is when we over expressed cyclins.

NOTE Confidence: 0.941363015833333

 $00:13:06.680 \rightarrow 00:13:09.284$  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
- $00:13:11.160 \longrightarrow 00:13:13.540$  one that expresses CDK 2 and cycling
- NOTE Confidence: 0.941363015833333
- $00:13:13.540 \rightarrow 00:13:16.266 \to So$  this is the cycling dependent
- NOTE Confidence: 0.941363015833333
- $00:13:16.266 \rightarrow 00:13:19.006$  kinase 2 and the cyclin here the cyclin
- NOTE Confidence: 0.941363015833333
- $00:13:19.006 \rightarrow 00:13:21.053$  E that activates it and separately
- NOTE Confidence: 0.941363015833333
- $00{:}13{:}21.053 \dashrightarrow 00{:}13{:}23.482$  the CDK four and it's cyclin CDK
- NOTE Confidence: 0.941363015833333
- $00:13:23.482 \rightarrow 00:13:25.778$  Cyclin D We call this guy 2E and
- NOTE Confidence: 0.941363015833333
- $00{:}13{:}25{.}778 \dashrightarrow 00{:}13{:}28{.}320$  this one 4D for obvious reasons.
- NOTE Confidence: 0.941363015833333
- $00:13:28.320 \longrightarrow 00:13:31.152$  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
- $00:13:35.572 \rightarrow 00:13:38.213$  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
- $00{:}13{:}43{.}240 \dashrightarrow 00{:}13{:}45{.}480$  basis now we had an erythroid bias.
- NOTE Confidence: 0.941363015833333
- $00:13:45.480 \rightarrow 00:13:47.678$  So the opposite with more cell cycle,
- NOTE Confidence: 0.941363015833333

 $00:13:47.680 \dashrightarrow 00:13:49.690$  more E fate specification and we

NOTE Confidence: 0.941363015833333

 $00{:}13{:}49.690 \dashrightarrow 00{:}13{:}52.586$  did not see this effect if we took

NOTE Confidence: 0.941363015833333

 $00:13:52.586 \rightarrow 00:13:54.806$  cells that were already MK committed

NOTE Confidence: 0.941363015833333

 $00:13:54.876 \rightarrow 00:13:57.000$  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

 $00:13:58.912 \rightarrow 00:14:00.357$  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

 $00:14:01.912 \rightarrow 00:14:03.745$  get more Erythroid. Why, how there?

NOTE Confidence: 0.941363015833333

 $00{:}14{:}03.745 \dashrightarrow 00{:}14{:}05.635$  We have a lot of ideas.

NOTE Confidence: 0.941363015833333

 $00:14:05.640 \rightarrow 00:14:07.082$  I'm going to show you that where

NOTE Confidence: 0.941363015833333

 $00{:}14{:}07{.}082 \dashrightarrow 00{:}14{:}08{.}782$  we are in terms of answering that

NOTE Confidence: 0.941363015833333

 $00{:}14{:}08{.}782 \dashrightarrow 00{:}14{:}10{.}318$  which is the runks one story

NOTE Confidence: 0.911813228695652

 $00{:}14{:}12.720 \dashrightarrow 00{:}14{:}14.763$  and we I'm not showing you the data but

NOTE Confidence: 0.911813228695652

 $00:14:14.763 \rightarrow 00:14:16.715$  we've shown that MEP actually cycle more

NOTE Confidence: 0.911813228695652

 $00:14:16.715 \rightarrow 00:14:18.839$  slowly than both Meg or Erythroid cells.

NOTE Confidence: 0.911813228695652

 $00:14:18.840 \longrightarrow 00:14:20.220$  So that's kind of an interesting

 $00:14:20.220 \longrightarrow 00:14:21.896$  concept that they have to speed up

NOTE Confidence: 0.911813228695652

 $00:14:21.896 \rightarrow 00:14:23.318$  whether they're going Meg or Erythroid,

NOTE Confidence: 0.911813228695652

 $00:14:23.320 \rightarrow 00:14:26.596$  it's just the degree to which they speed up.

NOTE Confidence: 0.911813228695652

 $00:14:26.600 \longrightarrow 00:14:28.472$  So I want to tell you about Runx 1.

NOTE Confidence: 0.911813228695652

 $00{:}14{:}28{.}480 \dashrightarrow 00{:}14{:}30{.}776$  Runx one also was revealed in our

NOTE Confidence: 0.911813228695652

 $00{:}14{:}30{.}776 \dashrightarrow 00{:}14{:}33{.}225$  single cell RNA seq data and then

NOTE Confidence: 0.911813228695652

 $00{:}14{:}33{.}225 \dashrightarrow 00{:}14{:}35{.}313$  subsequently in bulk RNA seq data.

NOTE Confidence: 0.911813228695652

 $00:14:35.320 \longrightarrow 00:14:37.070$  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

 $00:14:39.144 \rightarrow 00:14:40.719$  is likely regulating the genes,

NOTE Confidence: 0.911813228695652

 $00:14:40.720 \longrightarrow 00:14:43.191$  the change from MEP to MKP and

NOTE Confidence: 0.911813228695652

 $00:14:43.191 \longrightarrow 00:14:44.680$  from MEP to ERP,

NOTE Confidence: 0.911813228695652

 $00:14:44.680 \longrightarrow 00:14:46.955$  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.

 $00:14:51.120 \longrightarrow 00:14:53.424$  Ronx One was the predicted transcription

NOTE Confidence: 0.911813228695652

 $00:14:53.424 \rightarrow 00:14:56.039$  factor that would be regulating this.

NOTE Confidence: 0.911813228695652

 $00:14:56.040 \rightarrow 00:14:57.916$  Of the genes that are down regulated

NOTE Confidence: 0.911813228695652

 $00:14:57.916 \rightarrow 00:15:00.231$  from CMP to MEP and down regulated from

NOTE Confidence: 0.911813228695652

 $00:15:00.231 \rightarrow 00:15:02.360$  the MEP to the erythroid progenitor,

NOTE Confidence: 0.911813228695652

 $00{:}15{:}02{.}360 \dashrightarrow 00{:}15{:}04{.}592$  it was the number one ranked

NOTE Confidence: 0.911813228695652

 $00{:}15{:}04.592 \dashrightarrow 00{:}15{:}06.428$  transcription factor that was able

NOTE Confidence: 0.911813228695652

 $00:15:06.428 \rightarrow 00:15:08.516$  to regulate the target genes that

NOTE Confidence: 0.911813228695652

 $00:15:08.516 \rightarrow 00:15:09.560$  were differentially expressed.

NOTE Confidence: 0.911813228695652

 $00{:}15{:}09{.}560 \dashrightarrow 00{:}15{:}12{.}733$  It was also the number three potential

NOTE Confidence: 0.911813228695652

 $00{:}15{:}12.733 \dashrightarrow 00{:}15{:}14.491$  regulator of genes that are up

NOTE Confidence: 0.911813228695652

 $00:15:14.491 \longrightarrow 00:15:16.129$  regulated in the megacaryocyte fate

NOTE Confidence: 0.911813228695652

 $00:15:16.129 \rightarrow 00:15:17.545$  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

 $00{:}15{:}19{.}353 \dashrightarrow 00{:}15{:}21{.}691$  receptor and FLEA one which is a

NOTE Confidence: 0.911813228695652

 $00{:}15{:}21.691 \dashrightarrow 00{:}15{:}23.960$  known transcription factor that's

 $00:15:23.960 \rightarrow 00:15:28.880$  critical for megacaryois what we oops,

NOTE Confidence: 0.911813228695652

 $00:15:28.880 \dashrightarrow 00:15:30.116$  this is supposed to come next.

NOTE Confidence: 0.911813228695652

 $00:15:30.120 \longrightarrow 00:15:32.346$  What we did then was we over

NOTE Confidence: 0.911813228695652

 $00:15:32.346 \rightarrow 00:15:34.447$  expressed Runks 1 and when we do

NOTE Confidence: 0.911813228695652

 $00:15:34.447 \rightarrow 00:15:36.607$  that you can see that we actually

NOTE Confidence: 0.911813228695652

 $00:15:36.607 \rightarrow 00:15:39.427$  caused those bipotent cells to go

NOTE Confidence: 0.911813228695652

 $00{:}15{:}39{.}427 \dashrightarrow 00{:}15{:}41{.}185$  towards the megacaryocyte lineage.

NOTE Confidence: 0.911813228695652

 $00:15:41.185 \longrightarrow 00:15:43.600$  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

 $00{:}15{:}46{.}160 \dashrightarrow 00{:}15{:}48{.}472$  we could see the opposite effect where we

NOTE Confidence: 0.911813228695652

 $00:15:48.472 \longrightarrow 00:15:50.977$  see an increased in E fate specification

NOTE Confidence: 0.911813228695652

 $00{:}15{:}50{.}977 \dashrightarrow 00{:}15{:}53{.}510$  which really proved that the Runcs one

NOTE Confidence: 0.911813228695652

 $00:15:53.510 \rightarrow 00:15:57.640$  activity is promoting the MK fate in the MEP.

NOTE Confidence: 0.911813228695652

 $00:15:57.640 \longrightarrow 00:15:57.961$  However,

NOTE Confidence: 0.911813228695652

 $00{:}15{:}57{.}961 \dashrightarrow 00{:}16{:}00{.}529$  when we looked at Runcs One RNA and

 $00:16:00.529 \rightarrow 00:16:02.836$  protein levels in these three lineages,

NOTE Confidence: 0.911813228695652

 $00{:}16{:}02{.}840 \dashrightarrow 00{:}16{:}04{.}394$  the Meg erythroid progenitor and then the

NOTE Confidence: 0.911813228695652

 $00:16:04.394 \rightarrow 00:16:06.200$  Meg and the erythroid committed cells,

NOTE Confidence: 0.911813228695652

 $00:16:06.200 \longrightarrow 00:16:08.234$  there was no difference in either

NOTE Confidence: 0.911813228695652

 $00{:}16{:}08{.}234 \dashrightarrow 00{:}16{:}10{.}027$  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

 $00:16:11.922 \rightarrow 00:16:13.438$  the erythroid committed cells.

NOTE Confidence: 0.911813228695652

 $00:16:13.440 \longrightarrow 00:16:15.216$  Which told us it's not happening

NOTE Confidence: 0.911813228695652

 $00:16:15.216 \longrightarrow 00:16:16.400$  at the transcriptional level

NOTE Confidence: 0.911813228695652

 $00:16:16.449 \longrightarrow 00:16:17.757$  or the translational level.

NOTE Confidence: 0.911813228695652

 $00{:}16{:}17.760 \dashrightarrow 00{:}16{:}19.308$  It's probably post translational.

NOTE Confidence: 0.911813228695652

 $00:16:19.308 \longrightarrow 00:16:21.630$  So we started looking at post

NOTE Confidence: 0.911813228695652

 $00{:}16{:}21.698 \dashrightarrow 00{:}16{:}24.158$  translational modifications of Runx one.

NOTE Confidence: 0.911813228695652

 $00:16:24.160 \longrightarrow 00:16:26.106$  And one that has been heavily studied

NOTE Confidence: 0.911813228695652

 $00:16:26.106 \longrightarrow 00:16:27.720$  before is serine and three nine.

NOTE Confidence: 0.911813228695652

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

- NOTE Confidence: 0.911813228695652
- $00:16:30.186 \rightarrow 00:16:33.398$  to be necessary for its activation for
- NOTE Confidence: 0.911813228695652
- $00:16:33.398 \rightarrow 00:16:35.878$  its ability to activate transgenes.
- NOTE Confidence: 0.911813228695652
- $00:16:35.880 \longrightarrow 00:16:37.704$  So activate transcription.
- NOTE Confidence: 0.911813228695652
- 00:16:37.704 --> 00:16:38.920 I'm sorry.
- NOTE Confidence: 0.911813228695652
- $00:16:38.920 \longrightarrow 00:16:40.915$  What we did is we got antibodies
- NOTE Confidence: 0.911813228695652
- $00:16:40.915 \longrightarrow 00:16:42.697$  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
- $00:16:46.960 \longrightarrow 00:16:48.960$  he'd published this and pulled them out of
- NOTE Confidence: 0.911813228695652
- $00:16:48.960 \rightarrow 00:16:51.040$  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
- $00{:}16{:}51{.}960 \dashrightarrow 00{:}16{:}54{.}093$  and this is work that was done by two
- NOTE Confidence: 0.911813228695652
- $00{:}16{:}54.093 \dashrightarrow 00{:}16{:}56.320$  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

 $00:17:00.610 \longrightarrow 00:17:02.640$  student in the lab and she's really

NOTE Confidence: 0.821214104

 $00{:}17{:}02{.}709 \dashrightarrow 00{:}17{:}04{.}683$  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

 $00{:}17{:}12.605 \dashrightarrow 00{:}17{:}14.520$  one and phosphoserine runks 1.

NOTE Confidence: 0.821214104

 $00{:}17{:}14.520 \dashrightarrow 00{:}17{:}15.682$  And I'm going to show you data

NOTE Confidence: 0.821214104

 $00{:}17{:}15.682 \dashrightarrow 00{:}17{:}16.920$  for several of the phosphoserines.

NOTE Confidence: 0.821214104

 $00:17:16.920 \longrightarrow 00:17:18.120$  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

 $00{:}17{:}22.664 \dashrightarrow 00{:}17{:}25.838$  which is here in the Runks one protein.

NOTE Confidence: 0.821214104

 $00{:}17{:}25.840 \dashrightarrow 00{:}17{:}27.984$  And what she showed and this is just

NOTE Confidence: 0.821214104

 $00:17:27.984 \rightarrow 00:17:29.453$  representative data on the left and

NOTE Confidence: 0.821214104

 $00:17:29.453 \longrightarrow 00:17:31.148$  then graphed here on the right for

- NOTE Confidence: 0.821214104
- $00:17:31.148 \rightarrow 00:17:32.753$  multiple replicates is that either
- NOTE Confidence: 0.821214104
- $00:17:32.753 \longrightarrow 00:17:34.610$  with commitment to erythroid or
- NOTE Confidence: 0.821214104
- $00:17:34.610 \rightarrow 00:17:36.320$  megacarocyte fate specification,
- NOTE Confidence: 0.821214104
- $00{:}17{:}36{.}320 \dashrightarrow 00{:}17{:}40{.}759$  you see an increase in the phosphosurine
- NOTE Confidence: 0.821214104
- $00{:}17{:}40.759 \dashrightarrow 00{:}17{:}44.074$  levels of Bronx One and there's a
- NOTE Confidence: 0.821214104
- $00{:}17{:}44.074 \dashrightarrow 00{:}17{:}45.670$  significantly higher increase when
- NOTE Confidence: 0.821214104
- $00:17:45.738 \rightarrow 00:17:48.160$  you go to the Meg Fate specification.
- NOTE Confidence: 0.821214104
- $00:17:48.160 \rightarrow 00:17:51.420$  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
- $00{:}17{:}53{.}320 \dashrightarrow 00{:}17{:}56{.}312$  you see this increase and then a further
- NOTE Confidence: 0.821214104
- $00:17:56.312 \rightarrow 00:17:58.151$  significant increase between erythroid
- NOTE Confidence: 0.821214104
- 00:17:58.151 --> 00:18:00.195 progenitors and Meg progenitors.
- NOTE Confidence: 0.821214104
- $00:18:00.200 \rightarrow 00:18:01.718$  So that's just shown schematically here.
- NOTE Confidence: 0.821214104
- $00{:}18{:}01{.}720 \dashrightarrow 00{:}18{:}04{.}478$  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
  - 35

 $00:18:07.635 \rightarrow 00:18:10.557$  go up with when you go to Erythroid.

NOTE Confidence: 0.821214104

 $00:18:10.560 \longrightarrow 00:18:12.240$  So this is where we are.

NOTE Confidence: 0.821214104

 $00:18:12.240 \longrightarrow 00:18:14.424$  Is there a link now with the cell

NOTE Confidence: 0.821214104

 $00:18:14.424 \longrightarrow 00:18:16.160$  cycle data that I showed you?

NOTE Confidence: 0.821214104

 $00:18:16.160 \longrightarrow 00:18:17.648$  I'll just show you one example

NOTE Confidence: 0.821214104

 $00{:}18{:}17.648 \dashrightarrow 00{:}18{:}18.640$  to for this link.

NOTE Confidence: 0.821214104

 $00:18:18.640 \rightarrow 00:18:21.440$  The link is basically that the slowing

NOTE Confidence: 0.821214104

 $00:18:21.440 \rightarrow 00:18:24.560$  of the cell cycle requires Runcs 1.

NOTE Confidence: 0.821214104

 $00{:}18{:}24{.}560 \dashrightarrow 00{:}18{:}26{.}288$  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

 $00{:}18{:}27{.}943 \dashrightarrow 00{:}18{:}29{.}840$  don't get the Meg Fate specification.

NOTE Confidence: 0.821214104

 $00{:}18{:}29{.}840 \dashrightarrow 00{:}18{:}31{.}639$  They still go down the erythroid lineage.

NOTE Confidence: 0.821214104

 $00{:}18{:}31{.}640 \dashrightarrow 00{:}18{:}33{.}320$  But let me show that to you slowly.

NOTE Confidence: 0.821214104

 $00:18:33.320 \rightarrow 00:18:35.357$  So here's your control with the bipotent,

NOTE Confidence: 0.821214104

 $00{:}18{:}35{.}360 \dashrightarrow 00{:}18{:}37{.}320$  the erythroid only and the Meg only.

NOTE Confidence: 0.821214104

 $00{:}18{:}37{.}320 \dashrightarrow 00{:}18{:}39{.}561$  This is the effect of the Runx 1 inhibitor
$00:18:39.561 \rightarrow 00:18:41.398$  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

 $00:18:44.372 \longrightarrow 00:18:46.101$  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

 $00{:}18{:}47{.}960 \dashrightarrow 00{:}18{:}50{.}438$  And here I'm showing you the combination.

NOTE Confidence: 0.821214104

 $00{:}18{:}50{.}440 \dashrightarrow 00{:}18{:}52{.}820$  You do not see this increase in

NOTE Confidence: 0.821214104

 $00{:}18{:}52.820 \dashrightarrow 00{:}18{:}54.527$  Meg phase specification with ATRA

NOTE Confidence: 0.821214104

 $00{:}18{:}54{.}527 \dashrightarrow 00{:}18{:}56{.}381$  or with rapamycin in the presence

NOTE Confidence: 0.821214104

 $00:18:56.381 \longrightarrow 00:18:58.679$  of the inhibitor of the Ronx one.

NOTE Confidence: 0.821214104

 $00:18:58.680 \rightarrow 00:19:01.025$  Really suggesting that we have a link

NOTE Confidence: 0.821214104

 $00{:}19{:}01{.}025 \dashrightarrow 00{:}19{:}03{.}573$  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

 $00{:}19{:}05{.}423 \dashrightarrow 00{:}19{:}07{.}798$  and increased MK phase specification.

NOTE Confidence: 0.70433965

 $00:19:11.600 \rightarrow 00:19:12.638$  Oh, so I did include this,

 $00:19:12.640 \longrightarrow 00:19:13.599$  I wasn't sure if I'd show this.

NOTE Confidence: 0.70433965

 $00{:}19{:}13.600 \dashrightarrow 00{:}19{:}15.224$  So then we actually did prove that

NOTE Confidence: 0.70433965

 $00:19:15.224 \rightarrow 00:19:17.207$  if you slow the cell cycle like

NOTE Confidence: 0.70433965

 $00:19:17.207 \longrightarrow 00:19:18.747$  with the Pelvicyclib that actually

NOTE Confidence: 0.70433965

 $00{:}19{:}18{.}747 \dashrightarrow 00{:}19{:}20{.}192$  slowed the cell cycle and then

NOTE Confidence: 0.70433965

 $00:19:20.192 \longrightarrow 00:19:22.132$  you wash it out and then you show

NOTE Confidence: 0.70433965

 $00{:}19{:}22{.}132 \dashrightarrow 00{:}19{:}23{.}836$  the cells are Meg fate specified,

NOTE Confidence: 0.70433965

 $00:19:23.840 \longrightarrow 00:19:26.594$  you actually get increased levels of

NOTE Confidence: 0.70433965

 $00{:}19{:}26{.}594 \dashrightarrow 00{:}19{:}29{.}607$  phosphosurine runks one at both 276 and 303.

NOTE Confidence: 0.70433965

 $00{:}19{:}29{.}607 \dashrightarrow 00{:}19{:}31{.}490$  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

 $00:19:33.080 \rightarrow 00:19:35.432$  but the ratios suggest that we probably

NOTE Confidence: 0.70433965

 $00{:}19{:}35{.}432 \dashrightarrow 00{:}19{:}37{.}775$  have a higher percentage of the

NOTE Confidence: 0.70433965

 $00{:}19{:}37{.}775 \dashrightarrow 00{:}19{:}39{.}880$  total runks that is phosphorylated.

NOTE Confidence: 0.70433965

 $00{:}19{:}39{.}880 \dashrightarrow 00{:}19{:}43{.}136$  So that's our link for now with fate

NOTE Confidence: 0.70433965

 $00:19:43.136 \dashrightarrow 00:19:45.160$  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
- $00{:}19{:}49{.}899 \dashrightarrow 00{:}19{:}51{.}394$  the serines and three nines that
- NOTE Confidence: 0.70433965
- $00:19:51.461 \rightarrow 00:19:53.519$  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
- $00{:}19{:}54{.}560 \dashrightarrow 00{:}19{:}55{.}880$  when we overexpressed Runx one,
- NOTE Confidence: 0.70433965
- $00:19:55.880 \rightarrow 00:19:58.196$  we get more MK fate specification.
- NOTE Confidence: 0.70433965
- $00:19:58.200 \rightarrow 00:20:01.196$  What if we mutate these four residues,
- NOTE Confidence: 0.70433965
- $00{:}20{:}01{.}200 \dashrightarrow 00{:}20{:}03{.}520$  3 serines and one three nine
- NOTE Confidence: 0.70433965
- $00:20:03.520 \longrightarrow 00:20:05.840$  to alanine in that case?
- NOTE Confidence: 0.70433965
- $00{:}20{:}05{.}840 \dashrightarrow 00{:}20{:}08{.}078$  We didn't get quite no effect.
- NOTE Confidence: 0.70433965
- $00{:}20{:}08{.}080 \dashrightarrow 00{:}20{:}08{.}772$  We got some effect,
- NOTE Confidence: 0.70433965
- $00{:}20{:}08.772 \dashrightarrow 00{:}20{:}10.046$  but it was a less strong effect
- NOTE Confidence: 0.70433965
- $00{:}20{:}10.046 \dashrightarrow 00{:}20{:}10.996$  than in the wild type.
- NOTE Confidence: 0.70433965
- $00{:}20{:}11.000 \dashrightarrow 00{:}20{:}13.555$  And in contrast when we changed the
- NOTE Confidence: 0.70433965

 $00:20:13.555 \rightarrow 00:20:15.485$  serines and three nines to aspartic

NOTE Confidence: 0.70433965

 $00:20:15.485 \rightarrow 00:20:17.795$  acid which mimics the phospho serine,

NOTE Confidence: 0.70433965

 $00{:}20{:}17.800 \dashrightarrow 00{:}20{:}19.886$  so all of the overexpressed brunx one NOTE Confidence: 0.70433965

 $00:20:19.886 \rightarrow 00:20:22.558$  is pre in a pre phosphorylated state.

NOTE Confidence: 0.70433965

 $00{:}20{:}22{.}560 \dashrightarrow 00{:}20{:}25{.}283$  We got a far stronger effect with

NOTE Confidence: 0.70433965

 $00{:}20{:}25{.}283 \dashrightarrow 00{:}20{:}27{.}248$  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

 $00:20:29.710 \longrightarrow 00:20:31.570$  that this is really playing a

NOTE Confidence: 0.70433965

 $00{:}20{:}31{.}570 \dashrightarrow 00{:}20{:}33{.}720$  role in MK fate specification.

NOTE Confidence: 0.70433965

 $00:20:33.720 \longrightarrow 00:20:35.172$  In order to study this we

NOTE Confidence: 0.70433965

 $00{:}20{:}35{.}172 \dashrightarrow 00{:}20{:}36{.}840$  then used a cell line model.

NOTE Confidence: 0.70433965

 $00:20:36.840 \longrightarrow 00:20:38.600$  So human erythro leukemia

NOTE Confidence: 0.70433965

 $00{:}20{:}38.600 \dashrightarrow 00{:}20{:}40.521$  cells are an OK model.

NOTE Confidence: 0.70433965

 $00:20:40.521 \longrightarrow 00:20:42.256$  When you add TPA they

NOTE Confidence: 0.70433965

 $00{:}20{:}42.256 \dashrightarrow 00{:}20{:}44.360$  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
- $00:20:45.872 \rightarrow 00:20:47.517$  of go down the erythroid lineage.

 $00:20:47.520 \rightarrow 00:20:49.298$  Anyway it's the best system we have

NOTE Confidence: 0.70433965

 $00:20:49.298 \longrightarrow 00:20:51.251$  for looking at this and what we wanted

NOTE Confidence: 0.70433965

 $00:20:51.251 \rightarrow 00:20:53.160$  to do is over express the wild type,

NOTE Confidence: 0.70433965

 $00:20:53.160 \longrightarrow 00:20:55.600$  the 4A mutant that has the alanine mutations,

NOTE Confidence: 0.70433965

 $00{:}20{:}55{.}600 \dashrightarrow 00{:}20{:}57{.}693$  the 4D mutant with the aspartic acid

NOTE Confidence: 0.70433965

 $00{:}20{:}57.693 \dashrightarrow 00{:}21{:}00.059$  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

 $00{:}21{:}01{.}866 \dashrightarrow 00{:}21{:}03{.}556$  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

 $00:21:06.080 \longrightarrow 00:21:07.716$  without inducing these cells to

NOTE Confidence: 0.70433965

 $00{:}21{:}07{.}716 \dashrightarrow 00{:}21{:}09{.}606$  differentiate with TPA we just we

NOTE Confidence: 0.70433965

 $00{:}21{:}09.606 \dashrightarrow 00{:}21{:}11.920$  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

 $00{:}21{:}14.840 \dashrightarrow 00{:}21{:}16.835$  You can see that they already start

 $00:21:16.835 \rightarrow 00:21:18.800$  to mature down the Meg lineage

NOTE Confidence: 0.70433965

 $00:21:18.800 \longrightarrow 00:21:20.965$  just by over expressing this pre

NOTE Confidence: 0.70433965

 $00:21:20.965 \rightarrow 00:21:22.905$  phosphorylated Bronx one compared

NOTE Confidence: 0.70433965

 $00:21:22.905 \longrightarrow 00:21:26.400$  to the 4A or the wild type.

NOTE Confidence: 0.70433965

 $00{:}21{:}26{.}400 \dashrightarrow 00{:}21{:}28{.}010$  We then looked at gene expression changes

NOTE Confidence: 0.70433965

 $00{:}21{:}28.010 \dashrightarrow 00{:}21{:}29.879$  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

 $00{:}21{:}33{.}350 \dashrightarrow 00{:}21{:}35{.}121$  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

 $00{:}21{:}36{.}603 \dashrightarrow 00{:}21{:}38{.}494$  and this is just two duplicates of

NOTE Confidence: 0.70433965

 $00:21:38.494 \rightarrow 00:21:40.200$  each for the empty vector cells.

NOTE Confidence: 0.70433965

 $00:21:40.200 \longrightarrow 00:21:42.360$  For the cells that we're expressing

NOTE Confidence: 0.70433965

 $00:21:42.360 \longrightarrow 00:21:43.870$  4A that cannot be phosphorylated

NOTE Confidence: 0.70433965

 $00:21:43.870 \longrightarrow 00:21:45.078$  on this four residues,

NOTE Confidence: 0.70433965

 $00:21:45.080 \rightarrow 00:21:47.565$  the wild type and the 4D that's

- NOTE Confidence: 0.70433965
- $00:21:47.565 \rightarrow 00:21:48.275$  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
- $00:21:51.158 \rightarrow 00:21:53.270$  the glycoprotein 1B beta consistent with
- NOTE Confidence: 0.891751959166667
- $00{:}21{:}53{.}270 \dashrightarrow 00{:}21{:}55{.}932$  the increased CD 42 that we had seen when
- NOTE Confidence: 0.891751959166667
- $00{:}21{:}55{.}932 \dashrightarrow 00{:}21{:}58{.}200$  we looked at where is that Runx one bound.
- NOTE Confidence: 0.891751959166667
- $00{:}21{:}58{.}200 \dashrightarrow 00{:}22{:}00{.}223$  So the over expressed 4A4D and wild
- NOTE Confidence: 0.891751959166667
- $00:22:00.223 \rightarrow 00:22:02.390$  tape are all HA tagged and when we
- NOTE Confidence: 0.891751959166667
- $00{:}22{:}02{.}390 \dashrightarrow 00{:}22{:}04{.}659$ 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
- $00:22:06.280 \longrightarrow 00:22:07.920$  they all bind just fine.
- NOTE Confidence: 0.891751959166667
- $00{:}22{:}07{.}920 \dashrightarrow 00{:}22{:}10{.}524$  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
- $00{:}22{:}12{.}946 \dashrightarrow 00{:}22{:}15{.}240$  is not near those phosphocytes.
- NOTE Confidence: 0.891751959166667
- $00:22:15.240 \rightarrow 00:22:17.094$  But what it strongly suggests is
- NOTE Confidence: 0.891751959166667

 $00:22:17.094 \rightarrow 00:22:20.083$  that Runx one can bind but the post

NOTE Confidence: 0.891751959166667

 $00{:}22{:}20.083 \dashrightarrow 00{:}22{:}21.767$  translational modification is what's

NOTE Confidence: 0.891751959166667

 $00:22:21.767 \longrightarrow 00:22:24.158$  affecting its effect on transcription.

NOTE Confidence: 0.891751959166667

 $00:22:24.160 \longrightarrow 00:22:26.272$  And keep that in mind because I think

NOTE Confidence: 0.891751959166667

 $00{:}22{:}26{.}272 \dashrightarrow 00{:}22{:}28{.}566$  we we start to have clues now as

NOTE Confidence: 0.891751959166667

 $00{:}22{:}28.566 \dashrightarrow 00{:}22{:}30.558$  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

 $00{:}22{:}32{.}387 \dashrightarrow 00{:}22{:}34{.}326$  we combine the cut and run and the

NOTE Confidence: 0.891751959166667

 $00{:}22{:}34{.}326 \dashrightarrow 00{:}22{:}36{.}291$  RNA seek data that we have this group

NOTE Confidence: 0.891751959166667

 $00{:}22{:}36{.}291 \dashrightarrow 00{:}22{:}38{.}195$  of genes that are activated by both

NOTE Confidence: 0.891751959166667

 $00:22:38.200 \rightarrow 00:22:40.704$  wild type and 4D in the health cells

NOTE Confidence: 0.891751959166667

 $00:22:40.704 \longrightarrow 00:22:42.976$  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

 $00:22:43.552 \rightarrow 00:22:45.615$  the 4A mutant and those genes tend

NOTE Confidence: 0.891751959166667

 $00:22:45.615 \rightarrow 00:22:47.823$  to be genes that we know are very

NOTE Confidence: 0.891751959166667

 $00:22:47.823 \longrightarrow 00:22:49.198$  important in Meg maturation.

- NOTE Confidence: 0.891751959166667
- $00{:}22{:}49{.}200 \dashrightarrow 00{:}22{:}50{.}892$  So just consistent with what I

 $00{:}22{:}50.892 \dashrightarrow 00{:}22{:}52.838$  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

 $00{:}22{:}54{.}240 \dashrightarrow 00{:}22{:}55{.}657$  lot of data and a whole lot of

NOTE Confidence: 0.891751959166667

 $00{:}22{:}55.657 \dashrightarrow 00{:}22{:}56.960$  work on the cut and run data,

NOTE Confidence: 0.891751959166667

 $00{:}22{:}56{.}960 \dashrightarrow 00{:}23{:}00{.}120$  except to say that there really was no

NOTE Confidence: 0.891751959166667

 $00:23:00.120 \longrightarrow 00:23:01.777$  significant difference in binding of

NOTE Confidence: 0.891751959166667

 $00:23:01.777 \rightarrow 00:23:04.160$  the four a the wild type in the 4D.

NOTE Confidence: 0.891751959166667

 $00{:}23{:}04.160 \dashrightarrow 00{:}23{:}06.197$  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

 $00:23:08.040 \longrightarrow 00:23:09.000$  this is very recent data,

NOTE Confidence: 0.891751959166667

 $00:23:09.000 \longrightarrow 00:23:10.056$  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

 $00{:}23{:}13.440 \dashrightarrow 00{:}23{:}14.599$  the last few months.

 $00{:}23{:}14.600 \dashrightarrow 00{:}23{:}16.660$  Multiple kinases were published

NOTE Confidence: 0.891751959166667

 $00{:}23{:}16.660 \dashrightarrow 00{:}23{:}18.720$  that phosphorylate runks one,

NOTE Confidence: 0.891751959166667

 $00:23:18.720 \longrightarrow 00:23:20.390$  and problem is whether when

NOTE Confidence: 0.891751959166667

 $00:23:20.390 \rightarrow 00:23:22.480$  we knock down any of them,

NOTE Confidence: 0.891751959166667

 $00:23:22.480 \longrightarrow 00:23:27.439$  we had no loss of phosphorylation on Runx 1.

NOTE Confidence: 0.891751959166667

 $00:23:27.440 \longrightarrow 00:23:28.464$  So what's going on?

NOTE Confidence: 0.891751959166667

 $00:23:28.464 \rightarrow 00:23:30.920$  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

 $00:23:32.404 \longrightarrow 00:23:33.280$  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

 $00:23:38.240 \rightarrow 00:23:39.680$  all of the cycling dependent kinases,

NOTE Confidence: 0.891751959166667

 $00{:}23{:}39{.}680 \dashrightarrow 00{:}23{:}41{.}992$  and CDKS 1-2 and six had all been

NOTE Confidence: 0.891751959166667

 $00{:}23{:}41.992 \dashrightarrow 00{:}23{:}44.399$  proven to phosphorylate it in vitro.

NOTE Confidence: 0.891751959166667

 $00:23:44.400 \longrightarrow 00:23:45.640$  Similarly with the SIP,

NOTE Confidence: 0.891751959166667

 $00:23:45.640 \rightarrow 00:23:46.880$  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
- $00{:}23{:}48{.}427 \dashrightarrow 00{:}23{:}49{.}965$  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
- $00{:}23{:}54{.}091 \dashrightarrow 00{:}23{:}56{.}317$  these genes or inhibited them with
- NOTE Confidence: 0.891751959166667
- $00:23:56.320 \rightarrow 00:23:59.680$  with with very small molecules.
- NOTE Confidence: 0.891751959166667
- 00:23:59.680 --> 00:24:00.388 In fact,
- NOTE Confidence: 0.891751959166667
- $00:24:00.388 \longrightarrow 00:24:02.158$  if you what we found
- NOTE Confidence: 0.714086705
- $00:24:02.160 \longrightarrow 00:24:03.040$  is if if you
- NOTE Confidence: 0.90280320625
- 00:24:04.200 --> 00:24:05.610 inhibit CDK 9,
- NOTE Confidence: 0.90280320625
- $00{:}24{:}05{.}610 \dashrightarrow 00{:}24{:}07{.}960$  which is completely different CDK,
- NOTE Confidence: 0.90280320625
- $00:24:07.960 \longrightarrow 00:24:09.040$  that's when you lose it.
- NOTE Confidence: 0.90280320625
- $00:24:09.040 \longrightarrow 00:24:10.160$  So I'm going to show you first,
- NOTE Confidence: 0.90280320625
- $00{:}24{:}10.160 \dashrightarrow 00{:}24{:}11.280$  this is what happens when
- NOTE Confidence: 0.90280320625

 $00:24:11.280 \longrightarrow 00:24:12.640$  we inhibit CDK four or six.

NOTE Confidence: 0.90280320625

 $00{:}24{:}12{.}640 \dashrightarrow 00{:}24{:}14{.}116$  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

 $00:24:15.961 \longrightarrow 00:24:17.996$  be a kinase for Runx One.

NOTE Confidence: 0.90280320625

 $00{:}24{:}18.000 \dashrightarrow 00{:}24{:}19.460$  I previously showed you these

NOTE Confidence: 0.90280320625

 $00{:}24{:}19{.}460 \dashrightarrow 00{:}24{:}20{.}920$  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

 $00:24:22.440 \longrightarrow 00:24:23.820$  you actually get more

NOTE Confidence: 0.90280320625

 $00{:}24{:}23.820 \dashrightarrow 00{:}24{:}25.200$  phosphorylation of Runx One.

NOTE Confidence: 0.90280320625

 $00{:}24{:}25{.}200 \dashrightarrow 00{:}24{:}26{.}748$  Remember that was consistent with slowing

NOTE Confidence: 0.90280320625

 $00{:}24{:}26.748 \dashrightarrow 00{:}24{:}28.798$  the cell cycle more Runx 1 phosphorylation.

NOTE Confidence: 0.90280320625

 $00:24:28.800 \longrightarrow 00:24:31.640$  So CDK 6 is not the thing that's

NOTE Confidence: 0.90280320625

 $00:24:31.640 \rightarrow 00:24:33.560$  phosphorylating Runx One in our cells.

NOTE Confidence: 0.90280320625

 $00:24:33.560 \longrightarrow 00:24:36.560$  But when we inhibited CDK 9,

NOTE Confidence: 0.90280320625

 $00:24:36.560 \rightarrow 00:24:38.340$  which was another predicted kinase

NOTE Confidence: 0.90280320625

 $00:24:38.340 \longrightarrow 00:24:40.120$  that would phosphorylate these cells,

- NOTE Confidence: 0.90280320625
- $00:24:40.120 \rightarrow 00:24:41.878$  then we saw something really interesting.
- NOTE Confidence: 0.90280320625
- $00:24:41.880 \longrightarrow 00:24:43.692$  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
- $00{:}24{:}44.600 \dashrightarrow 00{:}24{:}46.784$  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
- $00{:}24{:}49.068 \dashrightarrow 00{:}24{:}51.840$  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
- $00:24:54.440 \longrightarrow 00:24:56.648$  but none of them is absolutely
- NOTE Confidence: 0.90280320625
- $00:24:56.648 \longrightarrow 00:24:58.120$  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
- $00:25:04.240 \longrightarrow 00:25:07.117$  and it induces degradation of CDK 9,
- NOTE Confidence: 0.90280320625
- $00{:}25{:}07{.}120 \dashrightarrow 00{:}25{:}09{.}024$  which I'm not showing you, but it does.
- NOTE Confidence: 0.90280320625
- $00:25:09.024 \rightarrow 00:25:11.232$  And when we added the Thou,
- NOTE Confidence: 0.90280320625

 $00:25:11.240 \longrightarrow 00:25:13.718$  we also got the loss of the

NOTE Confidence: 0.90280320625

 $00{:}25{:}13.718 \dashrightarrow 00{:}25{:}16.088$  phosphoserine 3O3 and phosphoserine 276.

NOTE Confidence: 0.90280320625

 $00{:}25{:}16.088 \dashrightarrow 00{:}25{:}18.680$  And when we added the Thou to the cells,

NOTE Confidence: 0.90280320625

 $00:25:18.680 \longrightarrow 00:25:19.760$  just as we had expected,

NOTE Confidence: 0.90280320625

 $00:25:19.760 \longrightarrow 00:25:22.960$  we got an erythroid bias to our MEP.

NOTE Confidence: 0.90280320625

 $00:25:22.960 \longrightarrow 00:25:23.646$  Really.

NOTE Confidence: 0.90280320625

 $00{:}25{:}23.646 \dashrightarrow 00{:}25{:}29.134$  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

 $00:25:32.760 \rightarrow 00:25:34.880$  Now for those of you who know what CDK 9 is,

NOTE Confidence: 0.90280320625

 $00:25:34.880 \rightarrow 00:25:35.768$  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

 $00{:}25{:}36{.}902 \dashrightarrow 00{:}25{:}38{.}200$  And I the answer is I don't know.

NOTE Confidence: 0.90280320625

 $00:25:38.200 \longrightarrow 00:25:40.360$  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

 $00:25:41.320 \longrightarrow 00:25:42.790$  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
- $00:25:45.613 \rightarrow 00:25:48.520$  transcriptional control apparatus.
- NOTE Confidence: 0.90280320625
- $00{:}25{:}48{.}520 \dashrightarrow 00{:}25{:}52{.}440$  It's part of activating RNA polymerase too,
- NOTE Confidence: 0.90280320625
- $00:25:52.440 \longrightarrow 00:25:54.408$  but in published data from years
- NOTE Confidence: 0.90280320625
- $00:25:54.408 \rightarrow 00:25:56.759$  ago that has never been explained.
- NOTE Confidence: 0.90280320625
- $00{:}25{:}56.760 \dashrightarrow 00{:}26{:}00.468$  Knock down the CDK 9 causes you to lose
- NOTE Confidence: 0.90280320625
- $00{:}26{:}00{.}468 \dashrightarrow 00{:}26{:}02{.}400$  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
- $00:26:04.840 \longrightarrow 00:26:07.114$  Runx One and Meg Fate specification
- NOTE Confidence: 0.90280320625
- $00{:}26{:}07{.}114 \dashrightarrow 00{:}26{:}09{.}998$  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
- $00{:}26{:}15.717 \dashrightarrow 00{:}26{:}17.659$  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
- $00{:}26{:}20.771 \dashrightarrow 00{:}26{:}22.348$  the transcriptional regulatory complex.
- NOTE Confidence: 0.90280320625
- $00{:}26{:}22{.}348 \dashrightarrow 00{:}26{:}25{.}456$  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

 $00:26:27.436 \longrightarrow 00:26:29.537$  answer to it yet is what is their

NOTE Confidence: 0.90280320625

 $00{:}26{:}29{.}537 \dashrightarrow 00{:}26{:}30{.}993$  differential binding as phosphosirring

NOTE Confidence: 0.90280320625

 $00:26:30.993 \rightarrow 00:26:33.359$  runks 1 to different target proteins.

NOTE Confidence: 0.90280320625

 $00{:}26{:}33{.}360 \dashrightarrow 00{:}26{:}35{.}268$  And we really want to do RNA seek and

NOTE Confidence: 0.90280320625

 $00{:}26{:}35{.}268 \dashrightarrow 00{:}26{:}37{.}302$  cut and run on these various different

NOTE Confidence: 0.90280320625

 $00{:}26{:}37{.}302 \dashrightarrow 00{:}26{:}39{.}190$  runks mutants in primary cells because

NOTE Confidence: 0.90280320625

 $00:26:39.190 \rightarrow 00:26:41.082$  everything I showed you for that

NOTE Confidence: 0.90280320625

 $00{:}26{:}41.082 \dashrightarrow 00{:}26{:}42.797$  so far was done in health cells.

NOTE Confidence: 0.90280320625

 $00:26:42.800 \longrightarrow 00:26:43.694$  And then really,

NOTE Confidence: 0.90280320625

 $00{:}26{:}43.694 \dashrightarrow 00{:}26{:}47.003$  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

 $00:26:51.600 \longrightarrow 00:26:52.464$  OK Act 3.

NOTE Confidence: 0.90280320625

 $00:26:52.464 \longrightarrow 00:26:54.480$  So act three is I showed you

NOTE Confidence: 0.914371405555556

 $00:26:54.565 \rightarrow 00:26:57.675$  that we get colonies and what we do is we

 $00:26:57.759 \rightarrow 00:27:00.797$  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

 $00:27:02.360 \rightarrow 00:27:04.115$  you say what colony types do we have,

NOTE Confidence: 0.914371405555556

 $00:27:04.120 \longrightarrow 00:27:06.682$  but we really then are not not,

NOTE Confidence: 0.914371405555556

 $00:27:06.682 \rightarrow 00:27:08.374$  don't know for sure what's happening

NOTE Confidence: 0.914371405555556

 $00:27:08.374 \longrightarrow 00:27:10.160$  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

 $00:27:12.242 \rightarrow 00:27:14.476$  proliferation in the cells before they

NOTE Confidence: 0.914371405555556

 $00{:}27{:}14.476 \dashrightarrow 00{:}27{:}16.840$  pick the erythroid fate and slower

NOTE Confidence: 0.914371405555556

 $00:27:16.840 \rightarrow 00:27:19.037$  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

 $00{:}27{:}20.160 \dashrightarrow 00{:}27{:}23.160$  We have to actually watch them

NOTE Confidence: 0.914371405555556

 $00{:}27{:}23.160 \dashrightarrow 00{:}27{:}25.160$  undergoing this fate specification.

NOTE Confidence: 0.914371405555556

 $00{:}27{:}25{.}160 \dashrightarrow 00{:}27{:}27{.}592$  So what Vanessa Scanlon lab and did in

NOTE Confidence: 0.914371405555556

 $00{:}27{:}27{.}592 \dashrightarrow 00{:}27{:}30{.}238$  my lab and Vanessa has now moved on.

 $00:27:30.240 \rightarrow 00:27:31.878$  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

 $00:27:33.918 \rightarrow 00:27:35.838$  University of Connecticut and what

NOTE Confidence: 0.914371405555556

 $00:27:35.838 \rightarrow 00:27:38.390$  she did is she developed a time lapse

NOTE Confidence: 0.914371405555556

 $00:27:38.460 \longrightarrow 00:27:41.370$  microscopy to watch individual human MEP

NOTE Confidence: 0.914371405555556

 $00{:}27{:}41{.}370 \dashrightarrow 00{:}27{:}43{.}800$  undergo fate specification in vitro.

NOTE Confidence: 0.91749533

 $00{:}27{:}46{.}920 \dashrightarrow 00{:}27{:}48{.}360$  So here's what she did.

NOTE Confidence: 0.91749533

 $00:27:48.360 \longrightarrow 00:27:49.836$  She took her facts, sorted MEP.

NOTE Confidence: 0.91749533

 $00{:}27{:}49{.}840 \dashrightarrow 00{:}27{:}51{.}624$  She put very few of them in a

NOTE Confidence: 0.91749533

 $00:27:51.624 \rightarrow 00:27:53.601$  very small volume in a in a plate

NOTE Confidence: 0.91749533

 $00{:}27{:}53{.}601 \dashrightarrow 00{:}27{:}55{.}398$  covered that and that in the same

NOTE Confidence: 0.91749533

 $00{:}27{:}55{.}398 \dashrightarrow 00{:}27{:}56{.}904$  semi solid medium that we use

NOTE Confidence: 0.91749533

 $00:27:56.904 \rightarrow 00:27:58.264$  for our colony forming essays.

NOTE Confidence: 0.91749533

 $00:27:58.264 \rightarrow 00:27:59.920$  But it has it's very flat.

NOTE Confidence: 0.91749533

 $00:27:59.920 \longrightarrow 00:28:01.656$  She puts a cover slip on top of

NOTE Confidence: 0.91749533

 $00:28:01.656 \rightarrow 00:28:03.316$  that puts it into the Viva view.

- NOTE Confidence: 0.91749533
- $00:28:03.320 \longrightarrow 00:28:04.840$  This is an Olympus apparatus

 $00:28:04.840 \longrightarrow 00:28:06.680$  we still have in the lab.

NOTE Confidence: 0.91749533

 $00:28:06.680 \longrightarrow 00:28:07.580$  It works beautifully.

NOTE Confidence: 0.91749533

 $00:28:07.580 \longrightarrow 00:28:09.080$  They don't make it anymore.

NOTE Confidence: 0.91749533

 $00{:}28{:}09{.}080 \dashrightarrow 00{:}28{:}10{.}916$  So for now we have it and then she

NOTE Confidence: 0.91749533

 $00{:}28{:}10.916 \dashrightarrow 00{:}28{:}12.681$  can watch these cells undergoing

NOTE Confidence: 0.91749533

 $00:28:12.681 \longrightarrow 00:28:14.561$  fate specification and add the

NOTE Confidence: 0.91749533

 $00:28:14.561 \longrightarrow 00:28:16.253$  antibodies towards the end of making

NOTE Confidence: 0.91749533

 $00{:}28{:}16{.}253 \dashrightarrow 00{:}28{:}18{.}140$  the movie so that the erythroid cells

NOTE Confidence: 0.91749533

 $00:28:18.140 \longrightarrow 00:28:20.310$  under are showing in red and the

NOTE Confidence: 0.91749533

 $00{:}28{:}20{.}310 \dashrightarrow 00{:}28{:}22{.}116$  megacuria sites are showing in green.

NOTE Confidence: 0.91749533

 $00{:}28{:}22{.}120 \dashrightarrow 00{:}28{:}24{.}514$  So here you have a bipotent colony,

NOTE Confidence: 0.91749533

 $00:28:24.520 \rightarrow 00:28:26.440$  a mega only colony and an erythroid colony.

NOTE Confidence: 0.91749533

 $00:28:26.440 \longrightarrow 00:28:28.156$  But they're all very flat because

NOTE Confidence: 0.91749533

 $00:28:28.156 \rightarrow 00:28:30.043$  we're looking at this and we're going

 $00:28:30.043 \longrightarrow 00:28:31.960$  to want to look at this over time.

NOTE Confidence: 0.91749533

 $00{:}28{:}31{.}960 \dashrightarrow 00{:}28{:}34{.}137$  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

 $00:28:39.040 \longrightarrow 00:28:40.918$  The little dots that color them,

NOTE Confidence: 0.91749533

 $00{:}28{:}40{.}920 \dashrightarrow 00{:}28{:}43{.}080$  we put those in, that's part of our analysis.

NOTE Confidence: 0.91749533

 $00:28:43.080 \longrightarrow 00:28:45.660$  So I don't have that pre dotted.

NOTE Confidence: 0.91749533

 $00:28:45.660 \rightarrow 00:28:47.480$  But anyway, so that's a single cell.

NOTE Confidence: 0.91749533

 $00{:}28{:}47{.}480 \dashrightarrow 00{:}28{:}49{.}316$  We're starting with a single MEP

NOTE Confidence: 0.91749533

 $00:28:49.320 \longrightarrow 00:28:50.982$  and then what you're going to

NOTE Confidence: 0.91749533

 $00:28:50.982 \rightarrow 00:28:52.896$  see is that that cell over time,

NOTE Confidence: 0.91749533

 $00:28:52.896 \longrightarrow 00:28:54.384$  and this is over the course

NOTE Confidence: 0.91749533

 $00:28:54.384 \longrightarrow 00:28:55.520$  of about seven days,

NOTE Confidence: 0.91749533

 $00{:}28{:}55{.}520 \dashrightarrow 00{:}28{:}56{.}720$  undergoes state specification.

NOTE Confidence: 0.91749533

00:28:56.720 --> 00:28:57.920 If it's blue,

NOTE Confidence: 0.91749533

 $00{:}28{:}57{.}920 \dashrightarrow 00{:}28{:}59{.}880$  it means that downstream of that cell

 $00:28:59.880 \rightarrow 00:29:02.079$  there are both Meg and Erythroid cells.

NOTE Confidence: 0.91749533

 $00{:}29{:}02.080 \dashrightarrow 00{:}29{:}02.788$  If it's red,

NOTE Confidence: 0.91749533

 $00:29:02.788 \longrightarrow 00:29:03.968$  it means everything downstream of

NOTE Confidence: 0.91749533

 $00:29:03.968 \rightarrow 00:29:05.717$  that is Erythroid and if it's green,

NOTE Confidence: 0.91749533

 $00:29:05.720 \longrightarrow 00:29:07.312$  it means everything downstream

NOTE Confidence: 0.91749533

 $00{:}29{:}07{.}312 \dashrightarrow 00{:}29{:}10{.}080$  of that is going to be Meg.

NOTE Confidence: 0.91749533

 $00:29:10.080 \longrightarrow 00:29:11.410$  And there are a lot of things

NOTE Confidence: 0.91749533

 $00:29:11.410 \longrightarrow 00:29:12.440$  that you can see here.

NOTE Confidence: 0.91749533

 $00:29:12.440 \longrightarrow 00:29:14.048$  One of them maybe you saw

NOTE Confidence: 0.91749533

 $00:29:14.048 \longrightarrow 00:29:15.120$  those streaky green lines,

NOTE Confidence: 0.91749533

 $00{:}29{:}15{.}120 \dashrightarrow 00{:}29{:}17{.}038$  the Meg progenitors move a whole lot

NOTE Confidence: 0.91749533

 $00{:}29{:}17.038 \dashrightarrow 00{:}29{:}19.119$  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

 $00{:}29{:}20.632 \dashrightarrow 00{:}29{:}21.777$  and whether it's relevant for

NOTE Confidence: 0.91749533

 $00{:}29{:}21.777 \dashrightarrow 00{:}29{:}23.359$  what's going on in the bone marrow.

00:29:23.360 --> 00:29:24.960 But what we do know is in the bone marrow,

NOTE Confidence: 0.91749533

 $00{:}29{:}24.960 \dashrightarrow 00{:}29{:}26.080$  people have looked at it,

NOTE Confidence: 0.91749533

 $00:29:26.080 \longrightarrow 00:29:28.555$  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

 $00:29:30.552 \longrightarrow 00:29:32.600$  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

 $00:29:34.168 \longrightarrow 00:29:35.582$  something to do with the fact

NOTE Confidence: 0.91749533

 $00:29:35.582 \longrightarrow 00:29:36.956$  that the Meg destined cell is

NOTE Confidence: 0.91749533

 $00{:}29{:}36{.}956 \dashrightarrow 00{:}29{:}38{.}253$  still quite motile and there are

NOTE Confidence: 0.91749533

 $00:29:38.253 \longrightarrow 00:29:39.159$  other things that you can see.

NOTE Confidence: 0.91749533

 $00:29:39.160 \longrightarrow 00:29:42.324$  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

 $00{:}29{:}44.127 \dashrightarrow 00{:}29{:}46.213$  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

 $00{:}29{:}47{.}840 \dashrightarrow 00{:}29{:}49{.}760$  but fewer and fewer of them.

NOTE Confidence: 0.91749533

 $00:29:49.760 \longrightarrow 00:29:51.720$  Some of the blue cells are still

- NOTE Confidence: 0.91749533
- $00:29:51.720 \longrightarrow 00:29:53.402$  here even pretty late when
- NOTE Confidence: 0.91749533
- $00:29:53.402 \longrightarrow 00:29:55.347$  the other ones still haven't
- NOTE Confidence: 0.91749533
- $00:29:55.347 \rightarrow 00:29:56.514$  undergone fate specification.
- NOTE Confidence: 0.91749533
- $00:29:56.520 \rightarrow 00:29:57.688$  When we analyze these,
- NOTE Confidence: 0.91749533
- $00{:}29{:}57.688 \dashrightarrow 00{:}29{:}59.920$  one of the first things we saw is.
- NOTE Confidence: 0.91749533
- $00{:}29{:}59{.}920 \dashrightarrow 00{:}30{:}01{.}384$  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
- $00{:}30{:}02{.}360 \dashrightarrow 00{:}30{:}04{.}106$  the red cells are erythroid committed
- NOTE Confidence: 0.91749533
- $00:30:04.106 \dashrightarrow 00:30:06.239$  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
- $00{:}30{:}07{.}108 \dashrightarrow 00{:}30{:}08{.}193$  I should probably say destined.
- NOTE Confidence: 0.957484911428572
- $00:30:08.200 \longrightarrow 00:30:09.516$  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
- $00{:}30{:}10.840 \dashrightarrow 00{:}30{:}12.160$  cells became at the end.
- NOTE Confidence: 0.957484911428572
- $00:30:12.160 \longrightarrow 00:30:13.456$  One thing you can see though
- NOTE Confidence: 0.957484911428572

 $00:30:13.456 \longrightarrow 00:30:14.760$  is that MEP self renewal,

NOTE Confidence: 0.957484911428572

 $00{:}30{:}14.760 \dashrightarrow 00{:}30{:}16.968$  this is not something any body had

NOTE Confidence: 0.957484911428572

 $00{:}30{:}16{.}968 \dashrightarrow 00{:}30{:}19{.}100$  ever known before and it was kind

NOTE Confidence: 0.957484911428572

 $00:30:19.100 \longrightarrow 00:30:20.420$  of questionable when you look at

NOTE Confidence: 0.957484911428572

 $00{:}30{:}20{.}420 \dashrightarrow 00{:}30{:}21{.}877$  the single cell RNA seek data.

NOTE Confidence: 0.957484911428572

 $00:30:21.880 \rightarrow 00:30:23.875$  If you remember we had this graduation,

NOTE Confidence: 0.957484911428572

 $00:30:23.880 \rightarrow 00:30:26.120$  I didn't know how long that graduation took.

NOTE Confidence: 0.957484911428572

 $00:30:26.120 \rightarrow 00:30:27.856$  Maybe cells just become an MEP and then

NOTE Confidence: 0.957484911428572

 $00{:}30{:}27.856 \dashrightarrow 00{:}30{:}29.558$  the next day they're mega erythroid.

NOTE Confidence: 0.957484911428572

 $00:30:29.560 \longrightarrow 00:30:31.429$  But here you can see that the

NOTE Confidence: 0.957484911428572

 $00:30:31.429 \longrightarrow 00:30:32.927$  bipotent cells can self renew

NOTE Confidence: 0.957484911428572

 $00:30:32.927 \longrightarrow 00:30:34.517$  and make more bipotent cells.

NOTE Confidence: 0.957484911428572

 $00:30:34.520 \rightarrow 00:30:36.140$  Sometimes where one bipotent cell

NOTE Confidence: 0.957484911428572

 $00:30:36.140 \longrightarrow 00:30:38.420$  makes 2 bipotent cells and times where

NOTE Confidence: 0.957484911428572

 $00:30:38.420 \rightarrow 00:30:40.534$  sometimes where it makes 1 bipotent cell

NOTE Confidence: 0.957484911428572

 $00:30:40.534 \rightarrow 00:30:42.679$  and one fate Destin cell unique fate.

 $00{:}30{:}42.680 \dashrightarrow 00{:}30{:}45.460$  And when we and this is just looking

NOTE Confidence: 0.957484911428572

 $00:30:45.460 \longrightarrow 00:30:47.113$  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

 $00:30:49.040 \rightarrow 00:30:50.642$  sometimes when we got we played

NOTE Confidence: 0.957484911428572

 $00{:}30{:}50{.}642 \dashrightarrow 00{:}30{:}52{.}718$  at MEP we got E only colonies.

NOTE Confidence: 0.957484911428572

 $00:30:52.720 \longrightarrow 00:30:53.875$  So this was another opportunity

NOTE Confidence: 0.957484911428572

 $00:30:53.875 \rightarrow 00:30:55.129$  for us to say, well,

NOTE Confidence: 0.957484911428572

 $00:30:55.129 \rightarrow 00:30:57.432$  is this different from when we plate

NOTE Confidence: 0.957484911428572

 $00{:}30{:}57{.}432 \dashrightarrow 00{:}30{:}59{.}371$  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

 $00:31:02.160 \longrightarrow 00:31:04.680$  And the answer is yes.

NOTE Confidence: 0.957484911428572

 $00{:}31{:}04{.}680 \dashrightarrow 00{:}31{:}06{.}731$  This is a sample tree from an

NOTE Confidence: 0.957484911428572

 $00{:}31{:}06{.}731 \dashrightarrow 00{:}31{:}09{.}014$  MEP that's going to undergo fate

NOTE Confidence: 0.957484911428572

 $00{:}31{:}09{.}014 \dashrightarrow 00{:}31{:}10{.}798$  specification down both lineages.

NOTE Confidence: 0.957484911428572

 $00{:}31{:}10.800 \dashrightarrow 00{:}31{:}12.888$  Here's one where it's going to

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

 $00:31:17.776 \rightarrow 00:31:19.360$  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

 $00:31:20.600 \rightarrow 00:31:22.846$  They make teeny tiny colonies and

NOTE Confidence: 0.957484911428572

 $00:31:22.846 \longrightarrow 00:31:24.476$  when we play erythroid progenitors,

NOTE Confidence: 0.957484911428572

 $00:31:24.480 \longrightarrow 00:31:27.098$  what we see is that they reach

NOTE Confidence: 0.957484911428572

 $00:31:27.098 \dashrightarrow 00:31:28.760$  this faster proliferation sooner.

NOTE Confidence: 0.957484911428572

 $00:31:28.760 \rightarrow 00:31:31.168$  So they really are downstream of this

NOTE Confidence: 0.957484911428572

 $00:31:31.168 \rightarrow 00:31:33.623$  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

 $00{:}31{:}35{.}602 \dashrightarrow 00{:}31{:}37{.}294$  and it doesn't speed up its

NOTE Confidence: 0.957484911428572

 $00:31:37.294 \rightarrow 00:31:38.680$  cell division quite so early.

NOTE Confidence: 0.935882898148148

 $00:31:40.920 \rightarrow 00:31:42.656$  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

 $00{:}31{:}44.759 \dashrightarrow 00{:}31{:}46.503$  were able to follow these cells for

 $00:31:46.503 \rightarrow 00:31:48.800$  up to 13 generations, a single cell,

NOTE Confidence: 0.935882898148148

 $00{:}31{:}48.800 \dashrightarrow 00{:}31{:}51.200$  what happens over 13 generations in

NOTE Confidence: 0.935882898148148

 $00:31:51.200 \rightarrow 00:31:53.399$  vitro and what you can see is expansion.

NOTE Confidence: 0.935882898148148

 $00:31:53.400 \rightarrow 00:31:55.596$  When one MEP makes 2 ME PS tends to

NOTE Confidence: 0.935882898148148

 $00{:}31{:}55{.}596 \dashrightarrow 00{:}31{:}57{.}995$  occur but one is that's where we started.

NOTE Confidence: 0.935882898148148

 $00{:}31{:}58{.}000 \dashrightarrow 00{:}31{:}59{.}590$  We only looked at colonies that

NOTE Confidence: 0.935882898148148

 $00:31:59.590 \longrightarrow 00:32:01.239$  were going to make both here.

NOTE Confidence: 0.935882898148148

 $00:32:01.240 \longrightarrow 00:32:03.408$  But what you can see is that you

NOTE Confidence: 0.935882898148148

 $00:32:03.408 \rightarrow 00:32:05.430$  really get MEP self renewal where

NOTE Confidence: 0.935882898148148

 $00:32:05.430 \longrightarrow 00:32:07.863$  you're going to get two expansion from

NOTE Confidence: 0.935882898148148

 $00{:}32{:}07{.}863 \dashrightarrow 00{:}32{:}10{.}600$  MET one MEP to two MEP for the 1st 3

NOTE Confidence: 0.935882898148148

 $00{:}32{:}10.600 \dashrightarrow 00{:}32{:}13.358$  divisions and then that gradually goes

NOTE Confidence: 0.935882898148148

 $00{:}32{:}13{.}358 \dashrightarrow 00{:}32{:}15{.}731$  away and by the 6th division you're

NOTE Confidence: 0.935882898148148

 $00:32:15.731 \rightarrow 00:32:18.077$  not getting one MEP making two MEP.

NOTE Confidence: 0.935882898148148

 $00{:}32{:}18.080 \dashrightarrow 00{:}32{:}19.725$  In contrast this maintenance division

 $00:32:19.725 \rightarrow 00:32:21.999$  where one daughter cells going to be a Meg,

NOTE Confidence: 0.935882898148148

 $00{:}32{:}22{.}000 \dashrightarrow 00{:}32{:}24{.}016$  an MEP and one is going to be fate

NOTE Confidence: 0.935882898148148

 $00{:}32{:}24.016 \dashrightarrow 00{:}32{:}25.852$  destined that starts to occur at

NOTE Confidence: 0.935882898148148

 $00:32:25.852 \rightarrow 00:32:27.412$  approximately the 4th generation and

NOTE Confidence: 0.935882898148148

 $00:32:27.469 \dashrightarrow 00:32:29.240$  that's what we have until the end.

NOTE Confidence: 0.935882898148148

 $00:32:29.240 \longrightarrow 00:32:30.598$  And with each time you have one

NOTE Confidence: 0.935882898148148

 $00:32:30.598 \rightarrow 00:32:31.640$  of these yellow divisions,

NOTE Confidence: 0.935882898148148

 $00:32:31.640 \rightarrow 00:32:34.020$  that's when one MEP makes 1 Erythroid

NOTE Confidence: 0.935882898148148

 $00:32:34.020 \rightarrow 00:32:36.439$  fate committed and one MK fate committed.

NOTE Confidence: 0.935882898148148

 $00:32:36.440 \longrightarrow 00:32:37.960$  That's going to be the end of the

NOTE Confidence: 0.935882898148148

 $00{:}32{:}37{.}960 \dashrightarrow 00{:}32{:}39{.}282$  line because we're not going to

NOTE Confidence: 0.935882898148148

 $00:32:39.282 \longrightarrow 00:32:39.954$  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

 $00:32:42.050 \rightarrow 00:32:43.758$  at the changes that occur over time,

NOTE Confidence: 0.935882898148148

 $00:32:43.760 \longrightarrow 00:32:46.232$  which ends up being highly relevant

NOTE Confidence: 0.935882898148148

 $00:32:46.232 \rightarrow 00:32:47.880$  for our predictive models.

 $00:32:47.880 \dashrightarrow 00:32:49.878$  What we wanted to do is come up with

NOTE Confidence: 0.935882898148148

 $00{:}32{:}49.878 \dashrightarrow 00{:}32{:}52.100$  a mathematical model that gave us the

NOTE Confidence: 0.935882898148148

 $00{:}32{:}52{.}100 \dashrightarrow 00{:}32{:}54{.}741$  outcome that we saw so that we could

NOTE Confidence: 0.935882898148148

 $00:32:54.741 \rightarrow 00:32:56.673$  understand the probability that a cell

NOTE Confidence: 0.935882898148148

 $00:32:56.680 \rightarrow 00:32:59.638$  would undergo a specific fate decision.

NOTE Confidence: 0.935882898148148

 $00{:}32{:}59{.}640 \dashrightarrow 00{:}33{:}01{.}472$  And this is work done by Everett Thompson

NOTE Confidence: 0.935882898148148

 $00:33:01.472 \rightarrow 00:33:03.838$  in my lab who's an amazing graduate student.

NOTE Confidence: 0.935882898148148

 $00{:}33{:}03{.}840 \dashrightarrow 00{:}33{:}06{.}837$  And what he realized is if he used a

NOTE Confidence: 0.935882898148148

 $00{:}33{:}06{.}837 \dashrightarrow 00{:}33{:}09{.}115$  Markov model of these cells that are

NOTE Confidence: 0.935882898148148

 $00:33:09.115 \rightarrow 00:33:11.314$  MEP that are expanding to make two

NOTE Confidence: 0.935882898148148

 $00:33:11.314 \rightarrow 00:33:13.300$  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

 $00:33:15.950 \longrightarrow 00:33:17.875$  versus these two maintenance divisions.

NOTE Confidence: 0.935882898148148

 $00{:}33{:}17.880 \dashrightarrow 00{:}33{:}20.240$  He could model the data that we got

NOTE Confidence: 0.935882898148148

 $00:33:20.240 \longrightarrow 00:33:22.862$  as long as he had that model change

- $00:33:22.862 \longrightarrow 00:33:23.514$  over time,
- NOTE Confidence: 0.935882898148148
- $00:33:23.520 \rightarrow 00:33:24.794$  which is consistent with what I just
- NOTE Confidence: 0.935882898148148
- $00:33:24.794 \longrightarrow 00:33:25.158$  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
- $00:33:27.547 \dashrightarrow 00:33:29.640$  probability that the MEP will self
- NOTE Confidence: 0.935882898148148
- $00{:}33{:}29{.}640 \dashrightarrow 00{:}33{:}30{.}639$  renew and expand.
- NOTE Confidence: 0.935882898148148
- $00:33:30.640 \longrightarrow 00:33:32.593$  So when he did that he got the data
- NOTE Confidence: 0.935882898148148
- $00:33:32.593 \longrightarrow 00:33:33.798$  that are plotted here.
- NOTE Confidence: 0.935882898148148
- $00:33:33.800 \rightarrow 00:33:36.579$  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
- $00:33:40.644 \rightarrow 00:33:44.359$  That is the data predicted by the model.
- NOTE Confidence: 0.935882898148148
- $00:33:44.360 \longrightarrow 00:33:47.920$  And then in the dotted line is the.
- NOTE Confidence: 0.935882898148148
- $00:33:47.920 \rightarrow 00:33:50.080$  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
- $00:33:50.346 \longrightarrow 00:33:52.474$  And the dotted line is the in blue

- NOTE Confidence: 0.935882898148148
- $00{:}33{:}52{.}474 \dashrightarrow 00{:}33{:}53{.}918$  is the observed data.
- NOTE Confidence: 0.935882898148148
- $00:33:53.920 \rightarrow 00:33:55.976$  So what you can see is we really
- NOTE Confidence: 0.935882898148148
- $00:33:55.976 \rightarrow 00:33:58.265$  are very closely modeling what the
- NOTE Confidence: 0.935882898148148
- $00:33:58.265 \rightarrow 00:34:01.200$  actual data are for the exhaustion,
- NOTE Confidence: 0.935882898148148
- $00:34:01.200 \rightarrow 00:34:02.568$  expansion, maintenance and maintenance.
- NOTE Confidence: 0.935882898148148
- $00:34:02.568 \longrightarrow 00:34:04.840$  The way to look at this is,
- NOTE Confidence: 0.935882898148148
- $00:34:04.840 \longrightarrow 00:34:05.470$  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
- $00:34:09.040 \longrightarrow 00:34:11.770$  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
- $00:34:16.226 \longrightarrow 00:34:17.630$  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
- $00{:}34{:}19.688 \dashrightarrow 00{:}34{:}22.000$  MK and a 17% chance of exhaustion.
- NOTE Confidence: 0.891759870909091

 $00:34:22.000 \longrightarrow 00:34:23.910$  Well, that kind of models our

NOTE Confidence: 0.891759870909091

 $00:34:23.910 \longrightarrow 00:34:26.300$  outcome in our CFU where we get about

NOTE Confidence: 0.891759870909091

 $00:34:26.300 \longrightarrow 00:34:28.336$  50% of the colonies have Mega and

NOTE Confidence: 0.891759870909091

 $00:34:28.336 \longrightarrow 00:34:30.231$  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

 $00:34:32.200 \dashrightarrow 00:34:35.288$  And then similarly you can look at another NOTE Confidence: 0.891759870909091

 $00:34:35.288 \rightarrow 00:34:37.040$  generation and get additional data.

NOTE Confidence: 0.915327742173913

 $00:34:39.120 \longrightarrow 00:34:41.010$  She Vanessa got a huge amount of

NOTE Confidence: 0.915327742173913

 $00{:}34{:}41.010 \dashrightarrow 00{:}34{:}43.424$  data out of this and I just want to

NOTE Confidence: 0.915327742173913

 $00:34:43.424 \rightarrow 00:34:45.320$  show you one other part of that.

NOTE Confidence: 0.915327742173913

 $00{:}34{:}45{.}320 \dashrightarrow 00{:}34{:}47{.}824$  And what she did is she analyzed the

NOTE Confidence: 0.915327742173913

 $00:34:47.824 \rightarrow 00:34:50.402$  length of the cell cycle and whether

NOTE Confidence: 0.915327742173913

 $00:34:50.402 \rightarrow 00:34:52.669$  that predicted output and it wasn't

NOTE Confidence: 0.915327742173913

 $00{:}34{:}52.669 \dashrightarrow 00{:}34{:}55.680$  as simple as we had hoped, but we did

NOTE Confidence: 0.915327742173913

 $00:34:55.680 \dashrightarrow 00:34:57.480$  get some statistically significant data.

NOTE Confidence: 0.915327742173913

 $00:34:57.480 \dashrightarrow 00:35:00.360$  The data that we got is that MEP

 $00:35:00.360 \rightarrow 00:35:03.576$  that are cycling slower are going

NOTE Confidence: 0.915327742173913

 $00:35:03.576 \longrightarrow 00:35:06.516$  to be the MK destined.

NOTE Confidence: 0.915327742173913

00:35:06.520 --> 00:35:07.660 Remember MEP cycling?

NOTE Confidence: 0.915327742173913

 $00:35:07.660 \rightarrow 00:35:09.560$  I have to remember exactly.

NOTE Confidence: 0.915327742173913

 $00{:}35{:}09{.}560 \dashrightarrow 00{:}35{:}10{.}840$  So there was no difference.

NOTE Confidence: 0.915327742173913

 $00:35:10.840 \dashrightarrow 00:35:12.800$  And this is where this was disappointing.

NOTE Confidence: 0.915327742173913

 $00:35:12.800 \rightarrow 00:35:15.760$  There was no difference in the cell cycle

NOTE Confidence: 0.915327742173913

 $00:35:15.760 \dashrightarrow 00:35:19.918$  interval between MEP and E destined cells.

NOTE Confidence: 0.915327742173913

 $00{:}35{:}19{.}920 \dashrightarrow 00{:}35{:}22{.}260$  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

 $00:35:24.131 \longrightarrow 00:35:26.357$  but that's not what we saw.

NOTE Confidence: 0.915327742173913

 $00{:}35{:}26{.}360 \dashrightarrow 00{:}35{:}27{.}122$  But we did.

NOTE Confidence: 0.915327742173913

 $00{:}35{:}27{.}122 \dashrightarrow 00{:}35{:}29{.}866$  What we did see is that once we and with

NOTE Confidence: 0.915327742173913

 $00{:}35{:}29.866 \dashrightarrow 00{:}35{:}31.959$  MK destined it was a little slower.

NOTE Confidence: 0.915327742173913

 $00{:}35{:}31{.}960 \dashrightarrow 00{:}35{:}34{.}039$  That's the point I wanted to make.

 $00:35:34.040 \longrightarrow 00:35:35.360$  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

 $00:35:37.068 \rightarrow 00:35:38.691$  slowly there was a very good chance

NOTE Confidence: 0.915327742173913

 $00:35:38.691 \rightarrow 00:35:40.599$  that it was going to be MK destined.

NOTE Confidence: 0.915327742173913

 $00:35:40.600 \rightarrow 00:35:42.553$  And then if you looked at the MKP themselves,

NOTE Confidence: 0.915327742173913

 $00{:}35{:}42{.}560 \dashrightarrow 00{:}35{:}42{.}920$  they are,

NOTE Confidence: 0.915327742173913

 $00:35:42.920 \rightarrow 00:35:44.360$  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

 $00:35:46.900 \rightarrow 00:35:48.490$  data was this MK Destined having

NOTE Confidence: 0.915327742173913

 $00:35:48.490 \longrightarrow 00:35:49.955$  a slightly slower cell cycle.

NOTE Confidence: 0.915327742173913

 $00:35:49.960 \dashrightarrow 00:35:51.640$  So not quite as clear as we would have liked,

NOTE Confidence: 0.915327742173913

 $00:35:51.640 \longrightarrow 00:35:53.440$  but that's what the data show

NOTE Confidence: 0.828415577142857

 $00:35:56.280 \longrightarrow 00:35:57.918$  this. So just this is this time

NOTE Confidence: 0.828415577142857

 $00{:}35{:}57{.}918 \dashrightarrow 00{:}35{:}59{.}864$  lapse imaging is now a tool in the

NOTE Confidence: 0.828415577142857

 $00:35:59.864 \rightarrow 00:36:01.400$  laboratory that we are enjoying using.

 $00:36:01.400 \longrightarrow 00:36:03.062$  If anybody wants to collaborate and

NOTE Confidence: 0.828415577142857

 $00:36:03.062 \rightarrow 00:36:04.839$  use this tool just let us know.

NOTE Confidence: 0.828415577142857

 $00:36:04.840 \longrightarrow 00:36:07.738$  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

 $00{:}36{:}13.680 \dashrightarrow 00{:}36{:}15.955$  wanted to tell you about expansion

NOTE Confidence: 0.828415577142857

 $00:36:15.955 \dashrightarrow 00:36:18.240$  microscopy to probe hematopoietic cells.

NOTE Confidence: 0.828415577142857

 $00:36:18.240 \rightarrow 00:36:20.120$  So what is expansion microscopy?

NOTE Confidence: 0.828415577142857

 $00:36:20.120 \longrightarrow 00:36:23.032$  This is a way of doing super

NOTE Confidence: 0.828415577142857

 $00:36:23.032 \rightarrow 00:36:25.398$  resolution microscopy using a confocal

NOTE Confidence: 0.828415577142857

 $00:36:25.398 \longrightarrow 00:36:26.992$  microscope and that really opens

NOTE Confidence: 0.828415577142857

 $00{:}36{:}26{.}992 \dashrightarrow 00{:}36{:}29{.}722$  up the door to all of those of us

NOTE Confidence: 0.828415577142857

 $00{:}36{:}29{.}722 \dashrightarrow 00{:}36{:}31{.}477$  who don't do electron microscopy.

NOTE Confidence: 0.828415577142857

 $00:36:31.480 \longrightarrow 00:36:33.480$  And even if you do do electron microscopy,

NOTE Confidence: 0.828415577142857

 $00:36:33.480 \longrightarrow 00:36:35.560$  you know it's very difficult to do any

00:36:35.560 --> 00:36:37.505 kind of immuno analysis because you're

NOTE Confidence: 0.828415577142857

 $00{:}36{:}37{.}505 \dashrightarrow 00{:}36{:}40{.}235$  really limited to the size of the gold

NOTE Confidence: 0.828415577142857

 $00:36:40.235 \longrightarrow 00:36:42.440$  balls that are attached to your antibody.

NOTE Confidence: 0.828415577142857

 $00:36:42.440 \longrightarrow 00:36:44.430$  So you maybe can look at two things at the

NOTE Confidence: 0.828415577142857

 $00:36:44.480 \longrightarrow 00:36:46.514$  same time and maybe can see where they are.

NOTE Confidence: 0.828415577142857

 $00{:}36{:}46{.}520 \dashrightarrow 00{:}36{:}48{.}936$  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

 $00:36:50.520 \longrightarrow 00:36:52.319$  not for every antigen with the expansion.

NOTE Confidence: 0.956286988888889

 $00:36:55.360 \rightarrow 00:36:57.475$  So this is just to get you guys interested,

NOTE Confidence: 0.956286988888889

 $00:36:57.480 \longrightarrow 00:36:58.960$  if you're not a pathologist

NOTE Confidence: 0.956286988888889

 $00:36:58.960 \rightarrow 00:37:00.440$  in looking at mega karyocytes,

NOTE Confidence: 0.956286988888889

 $00{:}37{:}00{.}440 \dashrightarrow 00{:}37{:}02{.}198$  they happen to be the most beautiful

NOTE Confidence: 0.956286988888889

 $00:37:02.200 \rightarrow 00:37:03.999$  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

 $00:37:05.760 \dashrightarrow 00:37:08.560$  very large, hence the name mega karyocyte.

NOTE Confidence: 0.956286988888889

 $00:37:08.560 \longrightarrow 00:37:10.160$  What we're looking at here
- NOTE Confidence: 0.956286988888889
- $00:37:10.160 \longrightarrow 00:37:12.220$  is a bunch of blood cells.
- NOTE Confidence: 0.956286988888889
- $00:37:12.220 \longrightarrow 00:37:13.995$  These are your normal neutrophils.
- NOTE Confidence: 0.956286988888889
- $00:37:14.000 \rightarrow 00:37:15.400$  You can see the size of their nucleus,
- NOTE Confidence: 0.956286988888889
- $00{:}37{:}15{.}400 \dashrightarrow 00{:}37{:}17{.}140$  it's about 8 microns and
- NOTE Confidence: 0.956286988888889
- $00:37:17.140 \longrightarrow 00:37:18.880$  this is a mega karyocyte.
- NOTE Confidence: 0.956286988888889
- $00:37:18.880 \longrightarrow 00:37:20.656$  It's a single cell.
- NOTE Confidence: 0.956286988888889
- $00:37:20.656 \rightarrow 00:37:23.817$  It's got this gigantic nucleus and a
- NOTE Confidence: 0.956286988888889
- $00:37:23.817 \rightarrow 00:37:26.840$  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
- $00:37:27.719 \rightarrow 00:37:29.477$  It's got the cell has divide,
- NOTE Confidence: 0.956286988888889
- $00:37:29.480 \longrightarrow 00:37:31.258$  the DNA has divided and the cell
- NOTE Confidence: 0.956286988888889
- $00:37:31.258 \longrightarrow 00:37:32.639$  has gotten bigger and bigger,
- NOTE Confidence: 0.956286988888889
- $00:37:32.640 \longrightarrow 00:37:34.080$  but the cell has not divided.
- NOTE Confidence: 0.956286988888889
- $00{:}37{:}34.080 \dashrightarrow 00{:}37{:}35.048$  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

 $00:37:37.360 \longrightarrow 00:37:40.560$  whatever, up to 128 clearly.

NOTE Confidence: 0.956286988888889

 $00{:}37{:}40{.}560 \dashrightarrow 00{:}37{:}42{.}716$  And then this part of this cell,

NOTE Confidence: 0.956286988888889

 $00:37:42.720 \longrightarrow 00:37:43.780$  which is super interesting

NOTE Confidence: 0.956286988888889

 $00:37:43.780 \longrightarrow 00:37:44.840$  and hard to describe,

NOTE Confidence: 0.956286988888889

 $00:37:44.840 \longrightarrow 00:37:46.640$  but you're about to see what it is.

NOTE Confidence: 0.956286988888889

 $00{:}37{:}46.640 \dashrightarrow 00{:}37{:}50.426$  It's not a single cell membrane

NOTE Confidence: 0.956286988888889

 $00:37:50.426 \longrightarrow 00:37:52.319$  surrounding a cytopus.

NOTE Confidence: 0.956286988888889

 $00:37:52.320 \longrightarrow 00:37:53.361$  Well, it is,

NOTE Confidence: 0.956286988888889

 $00{:}37{:}53{.}361 \dashrightarrow 00{:}37{:}55{.}443$  but the cell membrane is invaginated

NOTE Confidence: 0.956286988888889

 $00:37:55.443 \dashrightarrow 00:37:57.558$  all throughout that cytoplasm.

NOTE Confidence: 0.956286988888889

 $00:37:57.560 \longrightarrow 00:38:00.320$  And way you can see that is from this movie.

NOTE Confidence: 0.956286988888889

 $00:38:00.320 \longrightarrow 00:38:01.120$  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

 $00:38:03.720 \dashrightarrow 00:38:05.405$  who's an amazing mega karyocyte

NOTE Confidence: 0.956286988888889

 $00{:}38{:}05{.}405 \dashrightarrow 00{:}38{:}06{.}753$  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
- $00:38:11.640 \rightarrow 00:38:13.278$  And the thing that's amazing about
- NOTE Confidence: 0.956286988888889
- $00:38:13.278 \longrightarrow 00:38:15.306$  this movie is you can see that
- NOTE Confidence: 0.956286988888889
- $00:38:15.306 \rightarrow 00:38:16.776$  the cytoplasm is basically going
- NOTE Confidence: 0.956286988888889
- $00:38:16.776 \longrightarrow 00:38:18.722$  to unravel to release the pro
- NOTE Confidence: 0.956286988888889
- $00:38:18.722 \longrightarrow 00:38:20.352$  platelets that then become platelets.
- NOTE Confidence: 0.908491521428571
- $00:38:25.560 \rightarrow 00:38:27.996$  So all that membrane system was inside,
- NOTE Confidence: 0.908491521428571
- $00{:}38{:}28{.}000 \dashrightarrow 00{:}38{:}30{.}288$  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
- $00:38:33.030 \rightarrow 00:38:35.080$  and release these pro platelets.
- NOTE Confidence: 0.908491521428571
- $00{:}38{:}35{.}080 \dashrightarrow 00{:}38{:}37{.}796$  So yeah, it's a very cool movie.
- NOTE Confidence: 0.908491521428571
- $00:38:37.800 \rightarrow 00:38:40.710$  When people then look at megacary sites, they
- NOTE Confidence: 0.908491521428571
- $00{:}38{:}40.710 \dashrightarrow 00{:}38{:}43.160$  want to see that demarcation membrane system,
- NOTE Confidence: 0.908491521428571
- $00{:}38{:}43.160 \dashrightarrow 00{:}38{:}46.040$  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
- $00:38:48.280 \rightarrow 00:38:49.918$  So what is it, expansion microscopy?
- NOTE Confidence: 0.908491521428571
- $00:38:49.920 \rightarrow 00:38:53.760$  It's been developed in multiple laboratories.
- NOTE Confidence: 0.908491521428571
- $00{:}38{:}53.760 \dashrightarrow 00{:}38{:}55.056$  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
- $00{:}38{:}56{.}680 \dashrightarrow 00{:}38{:}57{.}916$  was the first to do it.
- NOTE Confidence: 0.908491521428571
- $00:38:57.920 \longrightarrow 00:38:59.432$  But these are the two people
- NOTE Confidence: 0.908491521428571
- $00{:}38{:}59{.}432 \dashrightarrow 00{:}39{:}00{.}440$  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
- $00{:}39{:}01{.}480 \dashrightarrow 00{:}39{:}02{.}444$  He's here at Yale.
- NOTE Confidence: 0.908491521428571
- $00{:}39{:}02{.}444 \dashrightarrow 00{:}39{:}04{.}148$  He does be autiful work with Pan XM
- NOTE Confidence: 0.908491521428571
- $00:39:04.148 \longrightarrow 00:39:06.308$  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
- $00:39:08.000 \rightarrow 00:39:12.319$  He has a different approach called magnify.
- NOTE Confidence: 0.908491521428571
- $00:39:12.320 \rightarrow 00:39:14.732$  And what you can see is that you take

00:39:14.732 --> 00:39:17.443 your cell and here we're just looking

NOTE Confidence: 0.908491521428571

 $00{:}39{:}17{.}443 \dashrightarrow 00{:}39{:}19{.}361$  at different the mitochondria and

NOTE Confidence: 0.908491521428571

 $00{:}39{:}19{.}361 \dashrightarrow 00{:}39{:}21{.}513$  the Golgi here in the cell and you

NOTE Confidence: 0.908491521428571

 $00:39:21.520 \rightarrow 00:39:25.078$  polymerize polyacrylamide gel into the cell,

NOTE Confidence: 0.908491521428571

 $00{:}39{:}25{.}080 \dashrightarrow 00{:}39{:}27{.}200$  hit it and it cross links with it.

NOTE Confidence: 0.908491521428571

 $00{:}39{:}27{.}200 \dashrightarrow 00{:}39{:}29{.}534$  You then expand that because there's

NOTE Confidence: 0.908491521428571

 $00:39:29.534 \rightarrow 00:39:32.199$  acrylamide in there and sodium acrylate.

NOTE Confidence: 0.908491521428571

 $00:39:32.200 \rightarrow 00:39:34.797$  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

 $00:39:35.514 \longrightarrow 00:39:36.228$  very absorptive.

NOTE Confidence: 0.908491521428571

 $00:39:36.228 \rightarrow 00:39:39.081$  So if you have sodium acrylate and then

NOTE Confidence: 0.908491521428571

 $00{:}39{:}39{.}081 \dashrightarrow 00{:}39{:}41{.}001$  you add water everything expands so

NOTE Confidence: 0.908491521428571

 $00{:}39{:}41.001 \dashrightarrow 00{:}39{:}43.311$  you get this huge expansion then what

NOTE Confidence: 0.908491521428571

 $00{:}39{:}43{.}311 \dashrightarrow 00{:}39{:}46{.}618$  they do in the boomers Dorf's lab is

NOTE Confidence: 0.908491521428571

 $00{:}39{:}46.618 \dashrightarrow 00{:}39{:}49.900$  they stop that get rid of the cross

 $00:39:49.900 \rightarrow 00:39:52.160$  linking re embedded and do it again.

NOTE Confidence: 0.908491521428571

 $00{:}39{:}52{.}160 \dashrightarrow 00{:}39{:}54{.}374$  So they can get up to 16 to 20

NOTE Confidence: 0.908491521428571

 $00:39:54.374 \longrightarrow 00:39:56.597$  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

 $00:39:58.608 \dashrightarrow 00:40:00.373$  expansion and I'll tell you about the

NOTE Confidence: 0.908491521428571

 $00{:}40{:}00{.}373 \dashrightarrow 00{:}40{:}02{.}438$  differences but we we do both in the lab.

NOTE Confidence: 0.908491521428571

 $00:40:02.440 \longrightarrow 00:40:04.752$  I mean the idea is you type take

NOTE Confidence: 0.908491521428571

 $00:40:04.752 \longrightarrow 00:40:06.838$  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

 $00:40:08.640 \rightarrow 00:40:12.167$  This is data from your Goersdorf's lab using

NOTE Confidence: 0.908491521428571

 $00{:}40{:}12.167 \dashrightarrow 00{:}40{:}15.600$  the Pan XM his two fold expansion approach.

NOTE Confidence: 0.908491521428571

 $00:40:15.600 \longrightarrow 00:40:18.240$  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

 $00:40:21.160 \longrightarrow 00:40:23.225$  So it gives you something that's very

NOTE Confidence: 0.908491521428571

 $00:40:23.225 \rightarrow 00:40:25.276$  similar to what you might see on EM.

NOTE Confidence: 0.908491521428571

 $00:40:25.280 \longrightarrow 00:40:26.880$  And you see this beautiful

- NOTE Confidence: 0.908491521428571
- $00:40:26.880 \longrightarrow 00:40:28.480$  Golgi apparatus in a cell.
- NOTE Confidence: 0.908491521428571
- $00:40:28.480 \longrightarrow 00:40:31.198$  This is just he LA cells.
- NOTE Confidence: 0.908491521428571
- $00:40:31.200 \rightarrow 00:40:33.642$  They can actually get antibodies to
- NOTE Confidence: 0.908491521428571
- $00{:}40{:}33.642 \dashrightarrow 00{:}40{:}36.433$  work that allow them to localize whether
- NOTE Confidence: 0.908491521428571
- $00:40:36.433 \longrightarrow 00:40:39.378$  a protein is on the outside or the
- NOTE Confidence: 0.908491521428571
- $00:40:39.378 \dashrightarrow 00:40:41.248$  inside of this of the mitochondria.
- NOTE Confidence: 0.908491521428571
- $00:40:41.248 \longrightarrow 00:40:43.670$  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
- $00:40:45.680 \longrightarrow 00:40:48.277$  it's on the inside of the mitochondria.
- NOTE Confidence: 0.908491521428571
- $00:40:48.280 \longrightarrow 00:40:49.624$  When they stain with anti Tom 20
- NOTE Confidence: 0.908491521428571
- $00:40:49.624 \rightarrow 00:40:51.288$  which is known to be on the outside
- NOTE Confidence: 0.908491521428571
- $00{:}40{:}51{.}288 \dashrightarrow 00{:}40{:}51{.}915$  of the mitochondria,
- NOTE Confidence: 0.908491521428571
- $00{:}40{:}51{.}920 \dashrightarrow 00{:}40{:}53{.}045$  you can see this different
- NOTE Confidence: 0.908491521428571
- $00{:}40{:}53.045 \dashrightarrow 00{:}40{:}54.106$  pattern and it's really,
- NOTE Confidence: 0.908491521428571
- $00:40:54.106 \longrightarrow 00:40:55.542$  really beautiful how you
- NOTE Confidence: 0.908491521428571

 $00:40:55.542 \longrightarrow 00:40:57.197$  can clearly see the Cox,

NOTE Confidence: 0.908491521428571

 $00:40:57.200 \longrightarrow 00:40:59.480$  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

 $00:41:02.120 \longrightarrow 00:41:02.463$  sorry.

NOTE Confidence: 0.908491521428571

 $00{:}41{:}02{.}463 \dashrightarrow 00{:}41{:}04{.}521$  So just be autiful imaging that we

NOTE Confidence: 0.908491521428571

 $00{:}41{:}04{.}521 \dashrightarrow 00{:}41{:}07{.}324$  want to be able to use in now in

NOTE Confidence: 0.908491521428571

 $00:41:07.324 \rightarrow 00:41:09.200$  mega carry sites and platelets.

NOTE Confidence: 0.908491521428571

 $00:41:09.200 \rightarrow 00:41:11.769$  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: 0.65870976777778

 $00:41:14.640 \rightarrow 00:41:17.079$  Pan XM that is in your Boomer source lab.

NOTE Confidence: 0.65870976777778

00:41:17.080 --> 00:41:20.750 And we really takes the best of both in some

NOTE Confidence: 0.65870976777778

00:41:20.835 --> 00:41:22.880 of our assays York Boomersdorf's approach.

NOTE Confidence: 0.65870976777778

 $00{:}41{:}22.880 \dashrightarrow 00{:}41{:}25.760$  The Pan XM gives you much better resolution.

NOTE Confidence: 0.65870976777778

 $00{:}41{:}25.760$  -->  $00{:}41{:}28.651$  No doubt you're getting 16X expansion and

NOTE Confidence: 0.65870976777778

 $00{:}41{:}28.651 \dashrightarrow 00{:}41{:}31.079$  you're really preserving morphology better.

NOTE Confidence: 0.65870976777778

 $00{:}41{:}31{.}080 \dashrightarrow 00{:}41{:}34{.}599$  However, it takes a lot of time and effort.

- NOTE Confidence: 0.65870976777778
- $00:41:34.600 \longrightarrow 00:41:36.520$  In contrast,
- NOTE Confidence: 0.65870976777778
- 00:41:36.520 --> 00:41:37.576 Yongshin's approach called Magnify,
- NOTE Confidence: 0.65870976777778
- $00:41:37.576 \longrightarrow 00:41:39.160$  just takes one to three days.
- NOTE Confidence: 0.65870976777778
- $00{:}41{:}39{.}160 \dashrightarrow 00{:}41{:}41{.}460$  It's less than an hour of hands on time per
- NOTE Confidence: 0.65870976777778
- $00:41:41.520 \rightarrow 00:41:43.879$  day and there's no special equipment needed.
- NOTE Confidence: 0.65870976777778
- 00:41:43.880 --> 00:41:45.205 You don't need this nitrogen
- NOTE Confidence: 0.65870976777778
- 00:41:45.205 --> 00:41:47.120 tank and you get less expansion,
- NOTE Confidence: 0.65870976777778
- $00:41:47.120 \longrightarrow 00:41:48.120$  but it's still quite beautiful.
- NOTE Confidence: 0.65870976777778
- 00:41:48.120 --> 00:41:49.338 So I'll show you some data
- NOTE Confidence: 0.65870976777778
- $00:41:49.338 \longrightarrow 00:41:50.440$  that we have for each.
- NOTE Confidence: 0.65870976777778
- $00{:}41{:}50{.}440 \dashrightarrow 00{:}41{:}53{.}716$  And this is not an expensive thing to do.
- NOTE Confidence: 0.65870976777778
- $00:41:53.720 \longrightarrow 00:41:56.216$  This is just a beautiful image
- NOTE Confidence: 0.65870976777778
- $00{:}41{:}56{.}216 \dashrightarrow 00{:}41{:}58{.}940$  that comes from the that we did in
- NOTE Confidence: 0.65870976777778
- $00{:}41{:}58{.}940 \dashrightarrow 00{:}42{:}00{.}000$  collaboration with your Brewers.
- NOTE Confidence: 0.65870976777778
- $00:42:00.000 \rightarrow 00:42:02.048$  Dorf's lab and your runs the imaging core
- NOTE Confidence: 0.65870976777778

 $00:42:02.048 \rightarrow 00:42:04.437$  for the Center of Excellence in Hematology.

NOTE Confidence: 0.65870976777778

 $00{:}42{:}04{.}440 \dashrightarrow 00{:}42{:}06{.}800$  And what you see here is a pan XM image.

NOTE Confidence: 0.65870976777778

 $00:42:06.800 \longrightarrow 00:42:09.638$  So that's the 16 fold increase,

NOTE Confidence: 0.65870976777778

 $00{:}42{:}09{.}640 \dashrightarrow 00{:}42{:}10{.}486$  20 fold increase.

NOTE Confidence: 0.65870976777778

 $00{:}42{:}10.486 \dashrightarrow 00{:}42{:}13.080$  And they pan stained it with the NHS Ester,

NOTE Confidence: 0.65870976777778

 $00:42:13.080 \dashrightarrow 00:42:16.560$  which stains all proteins and with M cling.

NOTE Confidence: 0.65870976777778

 $00:42:16.560 \rightarrow 00:42:18.360$  The nice thing about M cling is it bind,

NOTE Confidence: 0.65870976777778

 $00:42:18.360 \rightarrow 00:42:20.160$  you stain the cells before you expand them.

NOTE Confidence: 0.65870976777778

 $00:42:20.160 \longrightarrow 00:42:21.660$  It binds to membranes,

NOTE Confidence: 0.65870976777778

 $00:42:21.660 \longrightarrow 00:42:23.160$  it binds to lipids.

NOTE Confidence: 0.65870976777778

 $00{:}42{:}23.160 \dashrightarrow 00{:}42{:}26.200$  And this is allowing us to start to see this

NOTE Confidence: 0.65870976777778

 $00:42:26.279 \rightarrow 00:42:29.119$  invaginated membrane throughout the cell.

NOTE Confidence: 0.65870976777778

 $00:42:29.120 \longrightarrow 00:42:31.190$  And we're getting better and better

NOTE Confidence: 0.65870976777778

 $00:42:31.190 \longrightarrow 00:42:32.952$  images of this invagination that

NOTE Confidence: 0.65870976777778

 $00{:}42{:}32{.}952 \dashrightarrow 00{:}42{:}34{.}890$  tells that shows us the demarcation

NOTE Confidence: 0.65870976777778

 $00:42:34.890 \rightarrow 00:42:36.960$  membrane system of the megakaryocytes.

 $00:42:39.120 \rightarrow 00:42:40.674$  So here's another way of looking at

NOTE Confidence: 0.918802040909091

 $00:42:40.674 \rightarrow 00:42:41.640$  this demarcation membrane system.

NOTE Confidence: 0.918802040909091

 $00:42:41.640 \longrightarrow 00:42:43.809$  Now not with the M cling but just with

NOTE Confidence: 0.918802040909091

 $00:42:43.809 \longrightarrow 00:42:45.836$  the pan stain of all the proteins.

NOTE Confidence: 0.918802040909091

 $00:42:45.840 \rightarrow 00:42:48.367$  This is an electron microscopy image and

NOTE Confidence: 0.918802040909091

 $00{:}42{:}48{.}367 \dashrightarrow 00{:}42{:}51{.}640$  this is from our expanded whole bone marrow.

NOTE Confidence: 0.918802040909091

 $00:42:51.640 \rightarrow 00:42:53.600$  This is Mina Shu gave us this slide.

NOTE Confidence: 0.918802040909091

 $00:42:53.600 \longrightarrow 00:42:55.440$  So this is expanded bone

NOTE Confidence: 0.918802040909091

 $00{:}42{:}55{.}440 \dashrightarrow 00{:}42{:}57{.}280$  marrow from human FFPE tissue.

NOTE Confidence: 0.918802040909091

 $00:42:57.280 \longrightarrow 00:42:59.701$  And what you can see is that this PAN

NOTE Confidence: 0.918802040909091

 $00:42:59.701 \rightarrow 00:43:02.263$  XM really shows you the demarcation

NOTE Confidence: 0.918802040909091

 $00{:}43{:}02{.}263 \dashrightarrow 00{:}43{:}04{.}951$  membrane system similarly to what you

NOTE Confidence: 0.918802040909091

 $00{:}43{:}04{.}951 \dashrightarrow 00{:}43{:}07{.}315$  can see with the electron microscopy.

NOTE Confidence: 0.918802040909091

 $00{:}43{:}07{.}320 \dashrightarrow 00{:}43{:}08{.}740$  Here's another expanded thing.

NOTE Confidence: 0.918802040909091

 $00:43:08.740 \longrightarrow 00:43:10.515$  This is now from magnify,

 $00:43:10.520 \rightarrow 00:43:11.936$  showing that we have some antigens

NOTE Confidence: 0.918802040909091

 $00{:}43{:}11{.}936 \dashrightarrow 00{:}43{:}12{.}880$  that we can identify.

NOTE Confidence: 0.918802040909091

 $00:43:12.880 \longrightarrow 00:43:15.016$  We can identify CD 61 shown

NOTE Confidence: 0.918802040909091

 $00:43:15.016 \rightarrow 00:43:16.440$  in green and thrombospondin.

NOTE Confidence: 0.918802040909091

 $00{:}43{:}16{.}440 \dashrightarrow 00{:}43{:}19{.}240$  So these are megacary ocytes and

NOTE Confidence: 0.918802040909091

 $00{:}43{:}19{.}240 \dashrightarrow 00{:}43{:}21{.}974$  these green and red vesicles are

NOTE Confidence: 0.918802040909091

 $00:43:21.974 \rightarrow 00:43:23.456$  actually the granules that are going

NOTE Confidence: 0.918802040909091

 $00:43:23.456 \longrightarrow 00:43:24.918$  to become the platelet granules,

NOTE Confidence: 0.918802040909091

 $00:43:24.920 \longrightarrow 00:43:26.320$  the alpha granules that have

NOTE Confidence: 0.918802040909091

 $00:43:26.320 \rightarrow 00:43:27.720$  within them the thrombus bonded.

NOTE Confidence: 0.959589074

 $00:43:29.760 \longrightarrow 00:43:30.640$  And this is an image.

NOTE Confidence: 0.959589074

 $00:43:30.640 \rightarrow 00:43:32.476$  I just can't get it out of my mind.

NOTE Confidence: 0.959589074

 $00:43:32.480 \longrightarrow 00:43:33.680$  But we haven't seen this again,

NOTE Confidence: 0.959589074

 $00:43:33.680 \longrightarrow 00:43:34.640$  we haven't done this.

NOTE Confidence: 0.959589074

 $00:43:34.640 \longrightarrow 00:43:36.590$  Again, this is again the formal

NOTE Confidence: 0.959589074

 $00{:}43{:}36{.}590 \dashrightarrow 00{:}43{:}38{.}385$  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
- $00:43:44.000 \rightarrow 00:43:45.330$  And I can't get over this little
- NOTE Confidence: 0.959589074
- $00:43:45.330 \rightarrow 00:43:46.560$  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
- $00:43:48.639 \rightarrow 00:43:50.279$  where the invagination is happening,
- NOTE Confidence: 0.959589074
- $00:43:50.280 \longrightarrow 00:43:51.715$  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
- $00:43:52.770 \longrightarrow 00:43:53.920$  because this is a pathology,
- NOTE Confidence: 0.959589074
- $00{:}43{:}53{.}920 \dashrightarrow 00{:}43{:}55{.}798$  grand rounds and it's so beautiful.
- NOTE Confidence: 0.959589074
- $00:43:55.800 \rightarrow 00:43:57.396$  These are autofluorescent red blood cells.
- NOTE Confidence: 0.959589074
- $00{:}43{:}57{.}400 \dashrightarrow 00{:}43{:}59{.}824$  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
- $00{:}44{:}01{.}440 \dashrightarrow 00{:}44{:}03{.}665$  What what we've been quite
- NOTE Confidence: 0.959589074
- $00:44:03.665 \longrightarrow 00:44:05.640$  successful at is using this to
- NOTE Confidence: 0.959589074

 $00:44:05.640 \longrightarrow 00:44:07.260$  look at platelets and this is

NOTE Confidence: 0.959589074

 $00:44:07.322 \rightarrow 00:44:09.317$  work that was done by Max Carlino.

NOTE Confidence: 0.959589074

 $00:44:09.320 \longrightarrow 00:44:10.976$  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

 $00:44:12.360 \longrightarrow 00:44:13.928$  but he worked in my lab before

NOTE Confidence: 0.959589074

 $00{:}44{:}13{.}928 \dashrightarrow 00{:}44{:}15{.}782$  that and he worked on this

NOTE Confidence: 0.959589074

 $00:44:15.782 \rightarrow 00:44:17.278$  expansion microscopy on platelets.

NOTE Confidence: 0.959589074

 $00:44:17.280 \longrightarrow 00:44:19.848$  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

 $00:44:22.022 \rightarrow 00:44:23.702$  see that there are dense granules

NOTE Confidence: 0.959589074

 $00{:}44{:}23.702 \dashrightarrow 00{:}44{:}25.360$  and there are alpha granules.

NOTE Confidence: 0.959589074

 $00:44:25.360 \longrightarrow 00:44:27.097$  So I didn't mean to go to the next

NOTE Confidence: 0.959589074

 $00{:}44{:}27.097 \dashrightarrow 00{:}44{:}29.297$  one so quite so quickly at but you

NOTE Confidence: 0.959589074

 $00:44:29.297 \rightarrow 00:44:31.157$  need electron microscopy to see the details.

NOTE Confidence: 0.959589074

 $00{:}44{:}31{.}160 \dashrightarrow 00{:}44{:}34{.}238$  So what Max was able to do was expand

NOTE Confidence: 0.959589074

 $00:44:34.238 \rightarrow 00:44:36.436$  primary human platelets and then just

- NOTE Confidence: 0.959589074
- $00{:}44{:}36{.}436 \dashrightarrow 00{:}44{:}38{.}951$  this was just the pan staining with
- NOTE Confidence: 0.959589074
- $00{:}44{:}38{.}951 \dashrightarrow 00{:}44{:}41{.}436$  the protein stain you can see granules.
- NOTE Confidence: 0.959589074
- $00:44:41.440 \rightarrow 00:44:44.920$  Then he used antibody against thrombospondin.
- NOTE Confidence: 0.959589074
- 00:44:44.920 --> 00:44:45.186 Oops,
- NOTE Confidence: 0.959589074
- $00:44:45.186 \rightarrow 00:44:47.314$  it's supposed to be playing Oh well then
- NOTE Confidence: 0.959589074
- $00:44:47.314 \rightarrow 00:44:49.437$  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
- $00:44:51.080 \longrightarrow 00:44:52.856$  This is the thrombospondin
- NOTE Confidence: 0.959589074
- $00{:}44{:}52.856 \dashrightarrow 00{:}44{:}55.076$  which is in alpha granules.
- NOTE Confidence: 0.959589074
- $00{:}44{:}55{.}080 \dashrightarrow 00{:}44{:}57{.}366$  This is staining for tubulin which
- NOTE Confidence: 0.959589074
- $00:44:57.366 \longrightarrow 00:44:59.919$  is on the outside of platelets.
- NOTE Confidence: 0.959589074
- $00{:}44{:}59{.}920 \dashrightarrow 00{:}45{:}01{.}117$  So and just the way you expect,
- NOTE Confidence: 0.959589074
- $00{:}45{:}01{.}120 \dashrightarrow 00{:}45{:}03{.}115$  we can see this tubulin ring and
- NOTE Confidence: 0.959589074
- $00{:}45{:}03.115 \dashrightarrow 00{:}45{:}05.350$  this is showing you both the tubulin
- NOTE Confidence: 0.959589074
- $00:45:05.350 \longrightarrow 00:45:07.000$  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

 $00{:}45{:}09{.}255 \dashrightarrow 00{:}45{:}10{.}975$  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

 $00:45:13.137 \rightarrow 00:45:14.397$  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

 $00{:}45{:}16.416 \dashrightarrow 00{:}45{:}18.644$  left is some of the classic work

NOTE Confidence: 0.959589074

 $00:45:18.644 \rightarrow 00:45:20.304$  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

 $00{:}45{:}22{.}261 \dashrightarrow 00{:}45{:}25{.}336$  microscopy and they got about 50 such

NOTE Confidence: 0.959589074

 $00{:}45{:}25{.}336 \dashrightarrow 00{:}45{:}28{.}276$  granules per platelet on average.

NOTE Confidence: 0.959589074

 $00:45:28.280 \longrightarrow 00:45:29.568$  This is our data,

NOTE Confidence: 0.959589074

 $00:45:29.568 \rightarrow 00:45:31.178$  not counting them using electron

NOTE Confidence: 0.959589074

 $00:45:31.178 \rightarrow 00:45:32.801$  microscopy where you it's a huge

NOTE Confidence: 0.959589074

 $00:45:32.801 \rightarrow 00:45:35.186$  amount of time and effort to try to

NOTE Confidence: 0.959589074

 $00:45:35.186 \rightarrow 00:45:36.634$  get this three-dimensional microscopy.

 $00:45:36.640 \longrightarrow 00:45:39.076$  Here he can look at 151 platelets

NOTE Confidence: 0.959589074

 $00{:}45{:}39{.}076 \dashrightarrow 00{:}45{:}40{.}882$  in that stained slide that in the

NOTE Confidence: 0.959589074

 $00:45:40.882 \longrightarrow 00:45:42.582$  slide I just showed you and he can

NOTE Confidence: 0.959589074

 $00:45:42.582 \rightarrow 00:45:44.050$  say how many total granules are

NOTE Confidence: 0.959589074

 $00{:}45{:}44.050 \dashrightarrow 00{:}45{:}45.954$  there and how many granules are there

NOTE Confidence: 0.959589074

 $00{:}45{:}45{.}960 \dashrightarrow 00{:}45{:}47{.}520$  that have throm aspondon in them.

NOTE Confidence: 0.959589074

 $00{:}45{:}47{.}520 \dashrightarrow 00{:}45{:}48{.}968$  And he could see that there were a

NOTE Confidence: 0.959589074

 $00:45:48.968 \longrightarrow 00:45:50.307$  little bit more than 50 granules

NOTE Confidence: 0.959589074

 $00{:}45{:}50{.}307 \dashrightarrow 00{:}45{:}51{.}472$  on average per platelet looking

NOTE Confidence: 0.959589074

 $00:45:51.472 \longrightarrow 00:45:52.400$  very similar to this.

NOTE Confidence: 0.959589074

 $00:45:52.400 \longrightarrow 00:45:53.814$  And then he could even look at

NOTE Confidence: 0.959589074

 $00{:}45{:}53{.}814 \dashrightarrow 00{:}45{:}55{.}195$  what percentage of those platelets

NOTE Confidence: 0.959589074

 $00:45:55.195 \longrightarrow 00:45:55.919$  have thromaspondon.

NOTE Confidence: 0.959589074

 $00{:}45{:}55{.}920 \dashrightarrow 00{:}45{:}57{.}384$  So again something that I think

NOTE Confidence: 0.959589074

 $00:45:57.384 \longrightarrow 00:45:58.360$  can be useful clinically,

 $00:45:58.360 \rightarrow 00:46:02.200$  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

 $00:46:05.307 \rightarrow 00:46:07.431$  single cell RNA seek reveals MEP

NOTE Confidence: 0.8670297356

 $00{:}46{:}07{.}431 \dashrightarrow 00{:}46{:}09{.}954$  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

 $00:46:11.520 \rightarrow 00:46:13.220$  That cell cycle differences really

NOTE Confidence: 0.8670297356

 $00{:}46{:}13.220 \dashrightarrow 00{:}46{:}15.260$  seem to regulate MEP fate and

NOTE Confidence: 0.8670297356

 $00:46:15.260 \rightarrow 00:46:16.958$  we're trying to figure out how.

NOTE Confidence: 0.8670297356

 $00{:}46{:}16{.}960 \dashrightarrow 00{:}46{:}19{.}895$  One of the ways that seems to be working

NOTE Confidence: 0.8670297356

 $00{:}46{:}19.895 \dashrightarrow 00{:}46{:}22.520$  is through in a slower cycling cell

NOTE Confidence: 0.8670297356

 $00:46:22.520 \rightarrow 00:46:24.878$  there's more phosphoserine Ronx one,

NOTE Confidence: 0.8670297356

 $00{:}46{:}24.880 \dashrightarrow 00{:}46{:}26.280$  and that phosphoserine Ronx

NOTE Confidence: 0.8670297356

00:46:26.280 --> 00:46:27.680 1 activates Meg genes,

NOTE Confidence: 0.8670297356

 $00:46:27.680 \rightarrow 00:46:30.158$  so you get Meg fate specification.

NOTE Confidence: 0.8670297356

 $00{:}46{:}30{.}160 \dashrightarrow 00{:}46{:}32{.}242$  I showed you time lapse imaging

 $00:46:32.242 \longrightarrow 00:46:34.360$  that really showed at least that

NOTE Confidence: 0.8670297356

 $00:46:34.360 \rightarrow 00:46:35.776$  statistically significant slowing

NOTE Confidence: 0.8670297356

 $00:46:35.776 \longrightarrow 00:46:38.608$  of the cell cycle speed predicts

NOTE Confidence: 0.8670297356

 $00:46:38.608 \longrightarrow 00:46:40.824$  MK fade specification and that

NOTE Confidence: 0.8670297356

 $00:46:40.824 \longrightarrow 00:46:42.909$  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

 $00:46:45.107 \longrightarrow 00:46:47.237$  time with this Markov model.

NOTE Confidence: 0.8670297356

 $00:46:47.240 \longrightarrow 00:46:49.711$  And finally that we're very excited about

NOTE Confidence: 0.8670297356

 $00{:}46{:}49{.}711 \dashrightarrow 00{:}46{:}52{.}599$  the power of using expansion microscopy.

NOTE Confidence: 0.8670297356

 $00{:}46{:}52.600 \dashrightarrow 00{:}46{:}54.000$  I wanted to take a minute to tell

NOTE Confidence: 0.8670297356

 $00:46:54.000 \rightarrow 00:46:55.306$  you about the cooperative centers

NOTE Confidence: 0.8670297356

 $00{:}46{:}55{.}306 \dashrightarrow 00{:}46{:}56{.}518$  of excellence in hematology,

NOTE Confidence: 0.8670297356

 $00{:}46{:}56{.}520 \dashrightarrow 00{:}46{:}57{.}624$  which Karen already mentioned,

NOTE Confidence: 0.8670297356

 $00{:}46{:}57.624 \dashrightarrow 00{:}46{:}59.280$  but who listens to the intro.

NOTE Confidence: 0.8670297356

 $00:46:59.280 \longrightarrow 00:47:04.136$  So YCCEH is funded by the NIDDK.

 $00:47:04.136 \longrightarrow 00:47:06.768$  Yale is one of five such centers

NOTE Confidence: 0.8670297356

 $00{:}47{:}06.768 \dashrightarrow 00{:}47{:}08.599$  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

 $00:47:12.760 \longrightarrow 00:47:14.594$  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

 $00:47:16.533 \rightarrow 00:47:17.997$  including the expansion microscopy,

NOTE Confidence: 0.8670297356

00:47:18.000 - 00:47:20.960 the time lapse microscopy,

NOTE Confidence: 0.8670297356

 $00{:}47{:}20{.}960 \dashrightarrow 00{:}47{:}22{.}408$  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

 $00{:}47{:}24.646 \dashrightarrow 00{:}47{:}26.716$  as says and other across the country.

NOTE Confidence: 0.8670297356

 $00:47:26.720 \longrightarrow 00:47:28.360$  There's a metabolomics core for

NOTE Confidence: 0.8670297356

 $00:47:28.360 \longrightarrow 00:47:30.000$  any non malignant heme work

NOTE Confidence: 0.8670297356

 $00:47:30.064 \rightarrow 00:47:31.678$  that you're doing in at Utah.

NOTE Confidence: 0.8670297356

 $00:47:31.680 \longrightarrow 00:47:33.735$  There's an imaging core that

NOTE Confidence: 0.8670297356

 $00{:}47{:}33.735 \dashrightarrow 00{:}47{:}35.428$  does codecs in Indiana.

- NOTE Confidence: 0.8670297356
- $00:47:35.428 \longrightarrow 00:47:38.437$  You can get as many CD34 cells as
- NOTE Confidence: 0.8670297356
- $00:47:38.437 \rightarrow 00:47:40.111$  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
- $00{:}47{:}42.600 \dashrightarrow 00{:}47{:}45.210$  so do contact me if you want to be part
- NOTE Confidence: 0.8670297356
- $00:47:45.281 \longrightarrow 00:47:47.751$  of that or look it up at cceh dot IO.
- NOTE Confidence: 0.8670297356
- $00{:}47{:}47{.}760 \dashrightarrow 00{:}47{:}49{.}825$  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
- $00:47:57.522 \rightarrow 00:48:01.320$  of services at any one of the five cores,
- NOTE Confidence: 0.8670297356
- $00{:}48{:}01{.}320 \dashrightarrow 00{:}48{:}03{.}959$  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
- $00{:}48{:}05{.}864 \dashrightarrow 00{:}48{:}07{.}666$  submit it and we'll we review
- NOTE Confidence: 0.8670297356
- $00:48:07.666 \longrightarrow 00:48:09.184$  the monthly and then the Type
- NOTE Confidence: 0.8670297356

 $00:48:09.184 \rightarrow 00:48:10.656$  B grants are up to 70,000.

NOTE Confidence: 0.8670297356

 $00{:}48{:}10.656 \dashrightarrow 00{:}48{:}13.552$  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

 $00:48:15.880 \longrightarrow 00:48:17.612$  for non malignant hematology.

NOTE Confidence: 0.8670297356

00:48:17.612 $\operatorname{-->}$ 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<br/> NOTE Confidence: 0.8670297356

 $00:48:23.285 \longrightarrow 00:48:25.718$  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

 $00{:}48{:}28{.}440 \dashrightarrow 00{:}48{:}32{.}840$  So finally thank you to the lab,

NOTE Confidence: 0.8670297356

 $00{:}48{:}32{.}840 \dashrightarrow 00{:}48{:}34{.}250$  every body's pictured here and hopefully

NOTE Confidence: 0.8670297356

 $00:48:34.250 \longrightarrow 00:48:36.360$  I gave them credit as we went along.

NOTE Confidence: 0.8670297356

 $00:48:36.360 \longrightarrow 00:48:36.960$  Thanks so much.

NOTE Confidence: 0.666180874

 $00:48:45.640 \rightarrow 00:48:46.640$  This is open for questions.

NOTE Confidence: 0.5525023

 $00:48:50.480 \longrightarrow 00:48:50.720$  Yeah,

NOTE Confidence: 0.2580808375

 $00:48:52.800 \longrightarrow 00:48:55.680$  expansion by class is 34.

NOTE Confidence: 0.2580808375

00:48:55.680 --> 00:48:58.765 I'm wondering do you know if

 $00{:}48{:}58.765 \dashrightarrow 00{:}49{:}00.529$  that expansion material disrupt

NOTE Confidence: 0.2580808375

00:49:00.529 --> 00:49:02.999 like protein public interaction,

NOTE Confidence: 0.2580808375

 $00:49:03.000 \rightarrow 00:49:04.792$  did you try to see that colonization

NOTE Confidence: 0.2580808375

 $00:49:04.792 \longrightarrow 00:49:07.480$  of sort of things? Yeah. So

NOTE Confidence: 0.707747023333333

 $00{:}49{:}10.640 \dashrightarrow 00{:}49{:}12.398$  the answer is that proteins stay

NOTE Confidence: 0.707747023333333

 $00{:}49{:}12{.}400 \dashrightarrow 00{:}49{:}14{.}564$  intact and protein interactions they

NOTE Confidence: 0.707747023333333

 $00:49:14.564 \longrightarrow 00:49:16.520$  they're they say look Co localized,

NOTE Confidence: 0.707747023333333

 $00:49:16.520 \longrightarrow 00:49:18.424$  but I don't know if they stay

NOTE Confidence: 0.707747023333333

 $00:49:18.424 \longrightarrow 00:49:20.120$  negative if it's not prevailed.

NOTE Confidence: 0.707747023333333

 $00{:}49{:}20{.}120 \dashrightarrow 00{:}49{:}23{.}272$  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

 $00{:}49{:}27{.}931 \dashrightarrow 00{:}49{:}30{.}398$  visualize if you don't have extension.

NOTE Confidence: 0.707747023333333

 $00:49:30.400 \rightarrow 00:49:32.640$  So if you stain them after you've extended,

NOTE Confidence: 0.707747023333333

 $00:49:32.640 \longrightarrow 00:49:34.170$  you'll really be able to see

 $00:49:34.170 \longrightarrow 00:49:35.938$  that they were right next to each

NOTE Confidence: 0.707747023333333

 $00:49:35.938 \rightarrow 00:49:37.905$  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

 $00:49:39.772 \rightarrow 00:49:41.560$  blocking one another by being bad.

NOTE Confidence: 0.707747023333333

 $00{:}49{:}41{.}560 \dashrightarrow 00{:}49{:}43{.}840$  So people have done Co localization

NOTE Confidence: 0.707747023333333

 $00{:}49{:}43{.}840 \dashrightarrow 00{:}49{:}45{.}800$  studies with expansion that we ren't

NOTE Confidence: 0.707747023333333

 $00:49:45.800 \rightarrow 00:49:47.950$  feasible prior to having expansion.

NOTE Confidence: 0.707747023333333

 $00:49:47.950 \rightarrow 00:49:50.715$  But if you're asking other things when we

NOTE Confidence: 0.707747023333333

 $00{:}49{:}50.715 \dashrightarrow 00{:}49{:}53.079$  don't know what happens to DNA and RNA,

NOTE Confidence: 0.707747023333333

 $00:49:53.080 \rightarrow 00:49:55.117$  some people have gotten fish to work,

NOTE Confidence: 0.707747023333333

 $00:49:55.120 \longrightarrow 00:49:57.136$  but I don't really know what the

NOTE Confidence: 0.707747023333333

 $00{:}49{:}57{.}136 \dashrightarrow 00{:}49{:}59{.}104$  stretching does and what exactly gets

NOTE Confidence: 0.707747023333333

 $00:49:59.104 \rightarrow 00:50:01.426$  stretched at that tiny molecular level.

NOTE Confidence: 0.707747023333333

 $00{:}50{:}01{.}426 \dashrightarrow 00{:}50{:}03{.}920$  I've asked the same questions to the answer,

NOTE Confidence: 0.707747023333333

 $00:50:03.920 \longrightarrow 00:50:05.120$  but I'm not sure you know,

NOTE Confidence: 0.5280967575

 $00:50:06.600 \rightarrow 00:50:09.720$  with your increase in drugs on

- NOTE Confidence: 0.5280967575
- $00:50:09.720 \rightarrow 00:50:13.478$  causing the increase in accuracy.

 $00{:}50{:}13.480 \dashrightarrow 00{:}50{:}16.680$  Do you know that this later gets rise

NOTE Confidence: 0.5280967575

 $00:50:16.680 \rightarrow 00:50:18.560$  to functional increase in platelets?

NOTE Confidence: 0.749041935

 $00:50:20.880 \longrightarrow 00:50:23.348$  No. But what we do know,

NOTE Confidence: 0.749041935

 $00:50:23.348 \longrightarrow 00:50:24.770$  so our work was unique in

NOTE Confidence: 0.807950995517242

 $00{:}50{:}24.829 \dashrightarrow 00{:}50{:}26.549$  starting with the bipodent progenitor

NOTE Confidence: 0.807950995517242

 $00:50:26.549 \rightarrow 00:50:28.600$  and what we were always looking

NOTE Confidence: 0.807950995517242

 $00:50:28.600 \longrightarrow 00:50:30.752$  for is just which fake did it pick.

NOTE Confidence: 0.807950995517242

 $00:50:30.760 \longrightarrow 00:50:32.344$  And so you're right, we're only

NOTE Confidence: 0.807950995517242

 $00:50:32.344 \rightarrow 00:50:35.200$  looking like the first part of it,

NOTE Confidence: 0.807950995517242

 $00:50:35.200 \rightarrow 00:50:36.130$  but we didn't come up with

NOTE Confidence: 0.807950995517242

 $00:50:36.130 \longrightarrow 00:50:37.880$  rocks all by ourselves.

NOTE Confidence: 0.807950995517242

 $00{:}50{:}37{.}880 \dashrightarrow 00{:}50{:}40{.}078$  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

 $00{:}50{:}41{.}360 \dashrightarrow 00{:}50{:}43{.}436$  you have lower meds, lower platelets.

00:50:43.440 --> 00:50:44.560 If you over fresh rocks,

NOTE Confidence: 0.807950995517242

 $00{:}50{:}44{.}560 \dashrightarrow 00{:}50{:}46{.}240$  you have more meds and more platelets.

NOTE Confidence: 0.807950995517242

 $00{:}50{:}46{.}240 \dashrightarrow 00{:}50{:}47{.}902$  What wasn't known is where was

NOTE Confidence: 0.807950995517242

 $00:50:47.902 \longrightarrow 00:50:49.714$  that acting and that it might

NOTE Confidence: 0.807950995517242

 $00{:}50{:}49{.}714 \dashrightarrow 00{:}50{:}51{.}664$  be acting literally at the Fate

NOTE Confidence: 0.807950995517242

 $00:50:51.664 \rightarrow 00:50:52.920$  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

 $00:50:54.840 \longrightarrow 00:50:55.995$  but I can't tell you for sure.

NOTE Confidence: 0.4893077

 $00{:}50{:}56{.}400 \dashrightarrow 00{:}50{:}58{.}066$  I ask this because of our patients

NOTE Confidence: 0.4893077

 $00:50:58.066 \rightarrow 00:51:01.600$  with rocks one journal on mutation

NOTE Confidence: 0.4893077

 $00:51:01.600 \rightarrow 00:51:04.480$  and they in the bone marrow have the NOTE Confidence: 0.4893077

 $00{:}51{:}04{.}480 \dashrightarrow 00{:}51{:}07{.}000$  creation of abnormal and carrying sites,

NOTE Confidence: 0.4893077

 $00{:}51{:}07{.}000 \dashrightarrow 00{:}51{:}08{.}398$  but then they have Bronx the rapy.

NOTE Confidence: 0.73607370875

 $00{:}51{:}09{.}720 \dashrightarrow 00{:}51{:}13{.}040$  Those patients actually are

NOTE Confidence: 0.73607370875

 $00:51:13.040 \rightarrow 00:51:16.360$  hemisitis for inactivating mutation.

NOTE Confidence: 0.73607370875

 $00:51:16.360 \rightarrow 00:51:20.040$  They have decreased Bronx activity.

00:51:20.040 --> 00:51:22.630 So their mutant Bronx is either hypo

NOTE Confidence: 0.73607370875

 $00{:}51{:}22.630 \dashrightarrow 00{:}51{:}25.240$  functioning or not functioning at all.

NOTE Confidence: 0.73607370875

 $00:51:25.240 \rightarrow 00:51:27.760$  I wasn't aware then more plate, more megs.

NOTE Confidence: 0.73607370875

 $00:51:27.760 \rightarrow 00:51:30.096$  I know they have lower ploying megs because

NOTE Confidence: 0.73607370875

 $00:51:30.096 \rightarrow 00:51:32.307$  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

 $00:51:39.536 \longrightarrow 00:51:41.840$  I was wondering that the the

NOTE Confidence: 0.524295674615385

 $00{:}51{:}41{.}917 \dashrightarrow 00{:}51{:}44{.}407$  the common progenitor and in vitro,

NOTE Confidence: 0.524295674615385

 $00{:}51{:}44{.}407 \dashrightarrow 00{:}51{:}46{.}501$  the lineage commitment from the Detroit

NOTE Confidence: 0.524295674615385

 $00{:}51{:}46{.}501 \dashrightarrow 00{:}51{:}49{.}048$  and and Medicare is obviously driven by

NOTE Confidence: 0.524295674615385

 $00:51:49.048 \dashrightarrow 00:51:51.519$  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

 $00{:}51{:}54{.}790 \dashrightarrow 00{:}51{:}57{.}730$  vivo where those things are altered in

NOTE Confidence: 0.524295674615385

 $00:51:57.730 \longrightarrow 00:52:00.608$  a way that the the ratio you know the NOTE Confidence: 0.524295674615385

 $00:52:00.608 \rightarrow 00:52:01.952$  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

 $00:52:07.280 \rightarrow 00:52:11.600$  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

 $00:52:14.560 \longrightarrow 00:52:16.360$  we think it's truly lineage commitment

NOTE Confidence: 0.53965566

 $00{:}52{:}17{.}440 \dashrightarrow 00{:}52{:}19{.}040$  on a stochastic low because

NOTE Confidence: 0.807673904545455

 $00:52:19.040 \longrightarrow 00:52:20.021$  there's always probability.

NOTE Confidence: 0.807673904545455

 $00{:}52{:}20{.}021 \dashrightarrow 00{:}52{:}22{.}680$  We don't see that if we overspress rocks,

NOTE Confidence: 0.807673904545455

 $00:52:22.680 \rightarrow 00:52:23.556$  everything goes in it.

NOTE Confidence: 0.807673904545455

 $00{:}52{:}23.556 \dashrightarrow 00{:}52{:}25.480$  It's just some ability to go over throw it.

NOTE Confidence: 0.807673904545455

00:52:25.480 --> 00:52:27.664 In fact you need rocks one

NOTE Confidence: 0.807673904545455

 $00:52:27.664 \rightarrow 00:52:28.756$  for erythroid maturation.

NOTE Confidence: 0.807673904545455

 $00{:}52{:}28.760 \dashrightarrow 00{:}52{:}32.197$  So I think it's stochastic but biased

NOTE Confidence: 0.807673904545455

 $00:52:32.200 \longrightarrow 00:52:34.195$  that you know you have one ratio.

NOTE Confidence: 0.807673904545455

 $00:52:34.200 \rightarrow 00:52:35.604$  In the absence of over expressing

- NOTE Confidence: 0.807673904545455
- $00:52:35.604 \rightarrow 00:52:37.396$  wrongs you get a ratio that's very
- NOTE Confidence: 0.807673904545455
- $00{:}52{:}37{.}396 \dashrightarrow 00{:}52{:}38{.}998$  Meg biased when you over express
- NOTE Confidence: 0.807673904545455
- $00{:}52{:}38{.}998 \dashrightarrow 00{:}52{:}40{.}676$  wrongs and more Meg bias if you have
- NOTE Confidence: 0.737836598
- $00:52:40.920 \dashrightarrow 00:52:42.960$  normal. But in a normal progenitor
- NOTE Confidence: 0.737836598
- $00{:}52{:}42.960 \dashrightarrow 00{:}52{:}44.804$  what is the ratio of commitment
- NOTE Confidence: 0.737836598
- $00{:}52{:}44{.}804 \dashrightarrow 00{:}52{:}46{.}014$  towards something like a carrier
- NOTE Confidence: 0.737836598
- $00:52:46.014 \rightarrow 00:52:47.440$  site and the unit for itself?
- NOTE Confidence: 0.678241175
- $00{:}52{:}47{.}800 \dashrightarrow 00{:}52{:}49{.}494$  You're asking in vivo and I can
- NOTE Confidence: 0.678241175
- $00:52:49.494 \longrightarrow 00:52:51.180$  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
- $00{:}52{:}53.096 \dashrightarrow 00{:}52{:}56.440$  it seems that they're about equal and
- NOTE Confidence: 0.678241175
- $00{:}52{:}56{.}440 \dashrightarrow 00{:}52{:}58{.}680$  there's a there's a good reason for that.
- NOTE Confidence: 0.678241175
- $00:52:58.680 \rightarrow 00:53:01.230$  What happens downstream is the
- NOTE Confidence: 0.678241175
- $00:53:01.230 \longrightarrow 00:53:02.760$  erythroid progenitor proliferates
- NOTE Confidence: 0.678241175
- $00:53:02.760 \rightarrow 00:53:05.363$  log fold multiple times very quickly
- NOTE Confidence: 0.678241175

 $00:53:05.363 \longrightarrow 00:53:08.120$  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

 $00{:}53{:}13{.}910 \dashrightarrow 00{:}53{:}16{.}120$  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

 $00:53:21.210 \rightarrow 00:53:23.520$  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

 $00{:}53{:}24{.}948 \dashrightarrow 00{:}53{:}26{.}725$  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

 $00:53:27.720 \longrightarrow 00:53:29.000$  that's how it would go. And

NOTE Confidence: 0.508137418181818

 $00{:}53{:}29{.}000 \dashrightarrow 00{:}53{:}31{.}667$  we know if in older adults where

NOTE Confidence: 0.508137418181818

 $00:53:31.667 \longrightarrow 00:53:33.600$  there is minority buys that

NOTE Confidence: 0.508137418181818

 $00{:}53{:}33{.}600 \dashrightarrow 00{:}53{:}35{.}906$  that there is a a differential

NOTE Confidence: 0.508137418181818

 $00:53:35.906 \rightarrow 00:53:37.671$  response to this commitment between

 $00:53:37.671 \rightarrow 00:53:39.280$  megataryocytes and heart disease. I

NOTE Confidence: 0.90591115631579

 $00:53:39.280 \rightarrow 00:53:41.624$  don't know, I'd love to actually get access

NOTE Confidence: 0.90591115631579

 $00:53:41.624 \rightarrow 00:53:43.888$  to marrow from patients with different

NOTE Confidence: 0.90591115631579

 $00:53:43.888 \rightarrow 00:53:45.913$  diseases and that's been problematic.

NOTE Confidence: 0.90591115631579

 $00:53:45.920 \rightarrow 00:53:50.130$  We have looked at MPNS and in MPNS if they

NOTE Confidence: 0.90591115631579

 $00{:}53{:}50{.}238 \dashrightarrow 00{:}53{:}51{.}944$  have essential thrombocytosis then they

NOTE Confidence: 0.90591115631579

 $00{:}53{:}51{.}944 \dashrightarrow 00{:}53{:}55{.}288$  do tend to have a Meg bias to their MEP

NOTE Confidence: 0.90591115631579

 $00:53:55.288 \rightarrow 00:53:57.280$  and the opposite for polysychemia Vera.

NOTE Confidence: 0.90591115631579

 $00{:}53{:}57{.}280 \dashrightarrow 00{:}53{:}58{.}448$  But it's very subtle.

NOTE Confidence: 0.90591115631579

 $00{:}53{:}58{.}448 \dashrightarrow 00{:}54{:}01{.}444$  I think a lot of that is downstream of

NOTE Confidence: 0.90591115631579

 $00{:}54{:}01{.}444 \dashrightarrow 00{:}54{:}03{.}796$  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

 $00:54:06.862 \rightarrow 00:54:08.517$  They're acted the whole time.

NOTE Confidence: 0.90591115631579

 $00{:}54{:}08{.}520 \dashrightarrow 00{:}54{:}09{.}997$  So it's not going to just toggle

NOTE Confidence: 0.668145923333333

 $00:54:10.000 \longrightarrow 00:54:11.800$  it. Yeah. So

 $00:54:11.800 \longrightarrow 00:54:13.720$  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

 $00:54:18.401 \longrightarrow 00:54:20.600$  a lot of things in terms of their

NOTE Confidence: 0.595743156363636

 $00:54:20.600 \rightarrow 00:54:22.880$  cell cycling and their biases,

NOTE Confidence: 0.694387464117647

 $00{:}54{:}23.080 \dashrightarrow 00{:}54{:}25.984$  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

 $00{:}54{:}27{.}995 \dashrightarrow 00{:}54{:}30{.}277$  do have an increased risk of Chip

NOTE Confidence: 0.688225391

 $00{:}54{:}32{.}800 \dashrightarrow 00{:}54{:}34{.}408$  that might just be because they

NOTE Confidence: 0.688225391

 $00{:}54{:}34{.}408 \dashrightarrow 00{:}54{:}35{.}480$  have abnormal Hemato policies.

NOTE Confidence: 0.688225391

 $00{:}54{:}35{.}480 \dashrightarrow 00{:}54{:}37{.}223$  And so the few, the better cells

NOTE Confidence: 0.688225391

 $00:54:37.223 \rightarrow 00:54:39.198$  are the ones that are taking over.

NOTE Confidence: 0.688225391

 $00{:}54{:}39{.}200 \dashrightarrow 00{:}54{:}40{.}355$  But I I don't know for sure.

NOTE Confidence: 0.688225391

 $00:54:40.360 \longrightarrow 00:54:42.040$  It's a good question,

NOTE Confidence: 0.688225391

 $00:54:42.040 \longrightarrow 00:54:42.576$  really good question.

NOTE Confidence: 0.688225391

 $00:54:42.576 \longrightarrow 00:54:45.011$  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

 $00{:}54{:}46{.}488 \dashrightarrow 00{:}54{:}48{.}120$  that we can get access to cells.

NOTE Confidence: 0.36197081

 $00:54:52.880 \longrightarrow 00:54:53.560$  That question

NOTE Confidence: 0.52111016

 $00:54:55.640 \rightarrow 00:54:57.188$  hypothetically speaking, eventually

NOTE Confidence: 0.52111016

 $00{:}54{:}57{.}188 \dashrightarrow 00{:}55{:}00{.}800$  the red blood cells will be Euclided.

NOTE Confidence: 0.52111016

 $00{:}55{:}00{.}800 \dashrightarrow 00{:}55{:}02{.}736$  Is there part of the process that they

NOTE Confidence: 0.52111016

 $00{:}55{:}02.736 \dashrightarrow 00{:}55{:}04.825$  don't have to have a nucleus in the end

NOTE Confidence: 0.52111016

 $00:55:04.825 \rightarrow 00:55:06.680$  that allows them to proliferate so fast?

NOTE Confidence: 0.52111016

 $00{:}55{:}06{.}680 \dashrightarrow 00{:}55{:}09{.}508$  Is the ability of proliferation is is

NOTE Confidence: 0.52111016

 $00{:}55{:}09{.}508 \dashrightarrow 00{:}55{:}11{.}877$  reduced because they don't have to

NOTE Confidence: 0.52111016

 $00{:}55{:}11.877 \dashrightarrow 00{:}55{:}13.716$  maintain the full sort of nucleus.

NOTE Confidence: 0.52111016

 $00:55:13.716 \longrightarrow 00:55:15.480$  They can just go faster by being

NOTE Confidence: 0.52111016

 $00{:}55{:}15{.}531 \dashrightarrow 00{:}55{:}16{.}916$  more efficient in that way.

NOTE Confidence: 0.52111016

 $00:55:16.920 \longrightarrow 00:55:17.120$  They keep

NOTE Confidence: 0.427930538

 $00{:}55{:}17{.}200 \dashrightarrow 00{:}55{:}19{.}040$  it absolutely as well they're.

 $00:55:19.520 \rightarrow 00:55:20.920$  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

 $00:55:23.120 \longrightarrow 00:55:24.236$  as they as they go forward?

NOTE Confidence: 0.484321094285714

 $00:55:24.240 \rightarrow 00:55:26.039$  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

 $00:55:30.520 \rightarrow 00:55:33.480$  as these erythroid cells are matured,

NOTE Confidence: 0.484321094285714

 $00:55:33.480 \longrightarrow 00:55:34.440$  they're proliferating.

NOTE Confidence: 0.484321094285714

 $00:55:34.440 \rightarrow 00:55:37.320$  Again, matured that with the maturation,

NOTE Confidence: 0.484321094285714

 $00{:}55{:}37{.}320 \dashrightarrow 00{:}55{:}39{.}600$  the nucleus shuts down and the histones

NOTE Confidence: 0.484321094285714

 $00:55:39.600 \rightarrow 00:55:42.320$  get spit out. But prior to that,

NOTE Confidence: 0.484321094285714

 $00:55:42.320 \rightarrow 00:55:43.920$  when they're so proliferating,

NOTE Confidence: 0.484321094285714

 $00{:}55{:}43{.}920 \dashrightarrow 00{:}55{:}46{.}125$  I'm not aware of what's changing at

NOTE Confidence: 0.484321094285714

 $00{:}55{:}46.125 \dashrightarrow 00{:}55{:}47.960$  the chromatin level, but correct.

NOTE Confidence: 0.484321094285714

 $00{:}55{:}47{.}960 \dashrightarrow 00{:}55{:}49{.}308$  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
- $00:55:55.200 \rightarrow 00:55:56.970$  they express fewer and fewer genes
- NOTE Confidence: 0.484321094285714
- $00:55:56.970 \rightarrow 00:55:58.776$  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
- $00{:}56{:}00{.}480 \dashrightarrow 00{:}56{:}01{.}615$  like globins because it's going
- NOTE Confidence: 0.484321094285714
- $00:56:01.615 \longrightarrow 00:56:02.995$  to need all that globin for
- NOTE Confidence: 0.484321094285714
- $00{:}56{:}02.995 \dashrightarrow 00{:}56{:}04.195$  when it doesn't have a nucleus
- NOTE Confidence: 0.59440742
- $00{:}56{:}04{.}240 \dashrightarrow 00{:}56{:}05{.}900$  if they give them a timing advantage, if
- NOTE Confidence: 0.59440742
- $00{:}56{:}05{.}900 \dashrightarrow 00{:}56{:}07{.}520$  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
- $00:56:09.490 \longrightarrow 00:56:10.760$  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
- $00{:}56{:}17.680 \dashrightarrow 00{:}56{:}19.451$  so can you see any advantage to
- NOTE Confidence: 0.850209194814815
- $00{:}56{:}19{.}451 \dashrightarrow 00{:}56{:}21{.}314$  cycling faster or that you can cycle
- NOTE Confidence: 0.850209194814815

 $00:56:21.314 \rightarrow 00:56:23.233$  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

 $00:56:29.320 \longrightarrow 00:56:32.280$  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

 $00{:}56{:}36{.}160 \dashrightarrow 00{:}56{:}37{.}880$  I like it. Let me know when you

NOTE Confidence: 0.718126226153846

 $00:56:37.880 \longrightarrow 00:56:40.080$  have to go to that conference.

NOTE Confidence: 0.48513156

 $00:56:40.440 \longrightarrow 00:56:41.950$  So in addition to intrinsic

NOTE Confidence: 0.48513156

 $00:56:41.950 \rightarrow 00:56:43.760$  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

 $00{:}56{:}48.000 \dashrightarrow 00{:}56{:}49.666$  what about contribution from other

NOTE Confidence: 0.44606757

 $00:56:49.666 \rightarrow 00:56:51.515$  cell types either from other chromatic

NOTE Confidence: 0.44606757

 $00:56:51.515 \rightarrow 00:56:53.480$  cells and signals or thrombo cells.

NOTE Confidence: 0.831722017083333

 $00{:}56{:}55{.}720 \dashrightarrow 00{:}56{:}58{.}200$  We have looked really hard for other parts

NOTE Confidence: 0.831722017083333

 $00{:}56{:}58{.}200 \dashrightarrow 00{:}57{:}00{.}602$  of the micro environment that might affect

NOTE Confidence: 0.831722017083333

 $00:57:00.602 \dashrightarrow 00:57:03.837$  MEP fate and I did not include those data,

NOTE Confidence: 0.831722017083333

 $00{:}57{:}03{.}840 \dashrightarrow 00{:}57{:}05{.}775$  but we've done a lot of work and and
NOTE Confidence: 0.831722017083333

 $00:57:05.775 \dashrightarrow 00:57:07.360$  Vanessa's published quite a bit on it.

NOTE Confidence: 0.831722017083333

 $00{:}57{:}07{.}360 \dashrightarrow 00{:}57{:}09{.}961$  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

 $00:57:12.383 \rightarrow 00:57:14.399$  aware of erythropodent and thrombopodent.

NOTE Confidence: 0.831722017083333

 $00:57:14.400 \longrightarrow 00:57:16.230$  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

 $00{:}57{:}17{.}490 \dashrightarrow 00{:}57{:}19{.}270$  erythropodin making erythroid but

NOTE Confidence: 0.831722017083333

 $00:57:19.270 \longrightarrow 00:57:21.552$  they actually act super differently

NOTE Confidence: 0.831722017083333

 $00:57:21.552 \longrightarrow 00:57:22.878$  on different cells.

NOTE Confidence: 0.831722017083333

 $00:57:22.880 \longrightarrow 00:57:24.748$  Thrombopodin is the thrombopodin

NOTE Confidence: 0.831722017083333

 $00{:}57{:}24.748 \dashrightarrow 00{:}57{:}27.550$  receptor is is on hematopoietic stem

NOTE Confidence: 0.831722017083333

 $00{:}57{:}27.618 \dashrightarrow 00{:}57{:}29.916$  cell and all of those progenitors.

NOTE Confidence: 0.831722017083333

 $00{:}57{:}29{.}920 \dashrightarrow 00{:}57{:}31{.}400$  So they all need throm bopodin

NOTE Confidence: 0.831722017083333

 $00{:}57{:}31{.}400 \dashrightarrow 00{:}57{:}33{.}270$  and they're it's binding in the

NOTE Confidence: 0.831722017083333

 $00{:}57{:}33.270 \dashrightarrow 00{:}57{:}34.915$  middle of the thrombopod receptor.

NOTE Confidence: 0.831722017083333

 $00:57:34.920 \rightarrow 00:57:37.373$  When you get to the anti P level though,

NOTE Confidence: 0.831722017083333

 $00:57:37.373 \rightarrow 00:57:42.072$  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

 $00:57:44.320 \rightarrow 00:57:47.146$  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

 $00:57:50.600 \rightarrow 00:57:52.460$  What we thought then is if we add

NOTE Confidence: 0.831722017083333

 $00:57:52.460 \longrightarrow 00:57:54.255$  \*\*\*\*\*\* 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

 $00:57:55.520 \rightarrow 00:57:57.770$  No, what happened is when you

NOTE Confidence: 0.831722017083333

 $00:57:57.770 \longrightarrow 00:57:58.520$  remove thrombocodin,

NOTE Confidence: 0.831722017083333

 $00:57:58.520 \rightarrow 00:57:59.600$  I'm sorry saying that one.

NOTE Confidence: 0.831722017083333

 $00{:}57{:}59{.}600 \dashrightarrow 00{:}58{:}02{.}168$  When you remove throm bocodin you get

NOTE Confidence: 0.831722017083333

 $00{:}58{:}02.168 \dashrightarrow 00{:}58{:}05.119$  exactly the same ratio of colony types,

NOTE Confidence: 0.831722017083333

 $00:58:05.120 \longrightarrow 00:58:07.520$  but way fewer colonies and the

NOTE Confidence: 0.831722017083333

 $00:58:07.520 \longrightarrow 00:58:09.120$  colonies are teeny tiny.

NOTE Confidence: 0.831722017083333

 $00:58:09.120 \longrightarrow 00:58:10.737$  And when we look at the time

- NOTE Confidence: 0.831722017083333
- $00:58:10.737 \longrightarrow 00:58:11.720$  lapse microscopy of that,
- NOTE Confidence: 0.831722017083333
- $00:58:11.720 \rightarrow 00:58:14.114$  what we see is that the cells are dying.
- NOTE Confidence: 0.831722017083333
- $00:58:14.120 \longrightarrow 00:58:15.244$  So they're trying to,
- NOTE Confidence: 0.831722017083333
- $00:58:15.244 \rightarrow 00:58:16.368$  they're doing everything right
- NOTE Confidence: 0.831722017083333
- $00{:}58{:}16{.}368 \dashrightarrow 00{:}58{:}18{.}072$  at the beginning and then you
- NOTE Confidence: 0.831722017083333
- $00:58:18.072 \rightarrow 00:58:19.000$  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
- $00{:}58{:}21{.}248 \dashrightarrow 00{:}58{:}23{.}110$  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
- $00:58:29.960 \longrightarrow 00:58:30.659$  absolutely no difference
- NOTE Confidence: 0.831722017083333
- $00{:}58{:}30{.}659 \dashrightarrow 00{:}58{:}32{.}532$  in the ratio of output.
- NOTE Confidence: 0.831722017083333
- $00{:}58{:}32{.}532 \dashrightarrow 00{:}58{:}35{.}213$  We have seen an effect in this and
- NOTE Confidence: 0.831722017083333
- $00{:}58{:}35{.}213 \dashrightarrow 00{:}58{:}36{.}996$  this is part of Vanessa Scanlon's
- NOTE Confidence: 0.831722017083333

 $00:58:36.996 \longrightarrow 00:58:40.152$  work now in the lab that when she Co

NOTE Confidence: 0.831722017083333

 $00{:}58{:}40{.}152 \dashrightarrow 00{:}58{:}42{.}072$  cultures the cells with endothelial

NOTE Confidence: 0.831722017083333

 $00{:}58{:}42.072 \dashrightarrow 00{:}58{:}45.040$  cells then she also sees an an

NOTE Confidence: 0.831722017083333

 $00:58:45.040 \rightarrow 00:58:46.399$  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

 $00:58:48.450 \rightarrow 00:58:49.750$  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

 $00:58:51.880 \rightarrow 00:58:54.152$  What she wants to do now is very

NOTE Confidence: 0.831722017083333

 $00{:}58{:}54{.}152 \dashrightarrow 00{:}58{:}55{.}729$  methodically add different cell types

NOTE Confidence: 0.831722017083333

 $00{:}58{:}55{.}729 \dashrightarrow 00{:}58{:}57{.}927$  that are in the bone marrow micro

NOTE Confidence: 0.831722017083333

 $00{:}58{:}57{.}985 \dashrightarrow 00{:}59{:}00{.}703$  environment and determine how they affect

NOTE Confidence: 0.831722017083333

 $00:59:00.703 \rightarrow 00:59:02.515$  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

 $00:59:04.760 \rightarrow 00:59:05.796$  But that's as much as we know,

NOTE Confidence: 0.831722017083333

 $00:59:05.800 \dashrightarrow 00:59:07.354$  but it's definitely not TECO and ECO.

NOTE Confidence: 0.4187528975

 $00:59:08.640 \rightarrow 00:59:10.400$  Has she tried macrophages

- NOTE Confidence: 0.4187528975
- $00:59:10.400 \longrightarrow 00:59:12.800$  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
- $00:59:17.745 \longrightarrow 00:59:19.330$  she didn't have really good
- NOTE Confidence: 0.897041930769231
- $00{:}59{:}19{.}395 \dashrightarrow 00{:}59{:}20{.}598$  macrophages to use.
- NOTE Confidence: 0.897041930769231
- $00{:}59{:}20.600 \dashrightarrow 00{:}59{:}22.686$  We're much better in my lab at
- NOTE Confidence: 0.897041930769231
- $00{:}59{:}22.686 \dashrightarrow 00{:}59{:}24.240$  making urine macrophages than human.
- NOTE Confidence: 0.897041930769231
- $00:59:24.240 \rightarrow 00:59:26.240$  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
- $00:59:29.720 \longrightarrow 00:59:31.435$  very nice talk. So I think this
- NOTE Confidence: 0.53200364
- $00{:}59{:}31{.}440 \dashrightarrow 00{:}59{:}31{.}878$  is just a
- NOTE Confidence: 0.459704265
- $00:59:36.800 \dashrightarrow 00:59:40.920$  using the IPS cell, it's with the
- NOTE Confidence: 0.48968234
- $00{:}59{:}40{.}920 \dashrightarrow 00{:}59{:}42{.}439$  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
- $00:59:43.840 \longrightarrow 00:59:45.238$  that character. Is that true?
- NOTE Confidence: 0.569767385
- $00:59:46.560 \longrightarrow 00:59:48.200$  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

 $00:59:50.434 \rightarrow 00:59:52.640$  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

 $00:59:56.240 \longrightarrow 00:59:59.400$  progenitor cell line that is a it's a

NOTE Confidence: 0.569767385

 $00:59:59.400 \rightarrow 01:00:00.640$  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

 $01{:}00{:}03.146 \dashrightarrow 01{:}00{:}05.788$  from the patients make IPSC make this

NOTE Confidence: 0.569767385

 $01:00:05.788 \longrightarrow 01:00:08.920$  Meg cell line and basically it doesn't

NOTE Confidence: 0.569767385

 $01{:}00{:}08.920 \dashrightarrow 01{:}00{:}11.200$  look renty until you then induce

NOTE Confidence: 0.464620721428571

 $01:00:12.520 \rightarrow 01:00:16.720$  the movie show. When you see that, terrified,

NOTE Confidence: 0.640357305

 $01:00:18.840 \longrightarrow 01:00:21.720$  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

 $01:00:23.000 \rightarrow 01:00:24.400$  comes in the same size. Yeah,

NOTE Confidence: 0.4583143066666667

 $01{:}00{:}24.560 \dashrightarrow 01{:}00{:}25.916$  yeah, the arithmetics are always small

NOTE Confidence: 0.5726288466666667

 $01:00:25.920 \rightarrow 01:00:27.837$  and round and the bags get bigger and bigger

NOTE Confidence: 0.5829912225

 $01:00:27.840 \rightarrow 01:00:29.492$  and bigger and bigger. But when when

- NOTE Confidence: 0.5829912225
- $01{:}00{:}29{.}492 \dashrightarrow 01{:}00{:}31{.}360$  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
- $01:00:32.920 \longrightarrow 01:00:34.480$  about the same time that they
- NOTE Confidence: 0.75125650375
- $01:00:34.480 \longrightarrow 01:00:37.451$  express the C41. So by the time
- NOTE Confidence: 0.75125650375
- $01:00:37.451 \longrightarrow 01:00:38.319$  they're they're they're 41,
- NOTE Confidence: 0.75125650375
- $01:00:38.320 \longrightarrow 01:00:39.358$  they're they're kind of bigger now.
- NOTE Confidence: 0.81822744
- $01:00:42.240 \rightarrow 01:00:43.518$  We haven't looked at that carefully.
- NOTE Confidence: 0.81822744
- $01:00:43.520 \longrightarrow 01:00:45.956$  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
- $01{:}00{:}47.724 \dashrightarrow 01{:}00{:}48.880$  something different because they're
- NOTE Confidence: 0.81822744
- $01:00:48.880 \longrightarrow 01:00:50.320$  undergoing different nuclear changes.
- NOTE Confidence: 0.81822744
- $01{:}00{:}50{.}320 \dashrightarrow 01{:}00{:}51{.}120$  But we haven't looked that
- NOTE Confidence: 0.642686613333333
- $01{:}00{:}51{.}120 \dashrightarrow 01{:}00{:}52{.}278$  carefully. Thank you.
- NOTE Confidence: 0.4887288
- $01{:}00{:}55{.}080 \dashrightarrow 01{:}00{:}55{.}840$  One last question, the
- NOTE Confidence: 0.472317065
- $01:00:58.080 \rightarrow 01:01:01.560$  cell regulatory volume is it's super, super
- NOTE Confidence: 0.5321231366666667

 $01:01:04.000 \rightarrow 01:01:06.034$  tightly regulated and that carrier sets

NOTE Confidence: 0.5321231366666667

 $01{:}01{:}06.034 \dashrightarrow 01{:}01{:}08.080$  something very unique in that regard.

NOTE Confidence: 0.5321231366666667

01:01:08.080 --> 01:01:10.285 So I was wondering can you stall

NOTE Confidence: 0.5321231366666667

 $01:01:10.285 \longrightarrow 01:01:13.640$  that process and or carrier sets

NOTE Confidence: 0.5321231366666667

 $01:01:13.640 \longrightarrow 01:01:16.359$  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

 $01:01:18.640 \longrightarrow 01:01:20.656$  that be done or if that

NOTE Confidence: 0.815826193

01:01:20.656 --> 01:01:22.000 happens in any pathologies

NOTE Confidence: 0.82743574777778

01:01:24.560 $-\!\!>$ 01:01:27.108 it hasn't been done but many people

NOTE Confidence: 0.82743574777778

 $01{:}01{:}27.108 \dashrightarrow 01{:}01{:}29.398$  have tried not specifically with cell

NOTE Confidence: 0.82743574777778

01:01:29.398 --> 01:01:31.552 volume because many period sites can

NOTE Confidence: 0.82743574777778

 $01:01:31.552 \rightarrow 01:01:33.400$  make platelets as a 2N cell as 4,

NOTE Confidence: 0.82743574777778

 $01{:}01{:}33{.}400 \dashrightarrow 01{:}01{:}36{.}112$  N cell as 8, N as 16 and 32.

NOTE Confidence: 0.82743574777778

 $01:01:36.112 \longrightarrow 01:01:37.200$  So the question is,

NOTE Confidence: 0.82743574777778

 $01:01:37.200 \rightarrow 01:01:40.182$  what tells the men stop undergoing this

NOTE Confidence: 0.82743574777778

 $01:01:40.182 \rightarrow 01:01:43.678$  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: 0.82743574777778

01:01:46.656 --> 01:01:48.840 take the inside of a magnet making

NOTE Confidence: 0.82743574777778

 $01:01:48.909 \rightarrow 01:01:51.536$  platelets set up and Joe Battalion

NOTE Confidence: 0.82743574777778

 $01:01:51.536 \rightarrow 01:01:54.000$  and you inject it into a 2NA,

NOTE Confidence: 0.82743574777778

 $01:01:54.000 \rightarrow 01:01:55.626$  it'll make platelets.

NOTE Confidence: 0.82743574777778

 $01{:}01{:}55.626 \dashrightarrow 01{:}01{:}58.878$  So there's something that says go.

NOTE Confidence: 0.82743574777778

01:01:58.880 --> 01:02:00.100 And once you have it,

NOTE Confidence: 0.82743574777778

 $01:02:00.100 \longrightarrow 01:02:01.475$  you can transplant it into

NOTE Confidence: 0.82743574777778

 $01:02:01.475 \longrightarrow 01:02:02.759$  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

 $01:02:04.776 \longrightarrow 01:02:06.119$  shock to a melancharyocyte.

NOTE Confidence: 0.630230093846154

01:02:06.120 $\operatorname{-->}$ 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

 $01:02:17.280 \longrightarrow 01:02:18.200$  you. Next.