

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

FLUIDIGM, CORP.,
Petitioner,

v.

THE BOARD OF TRUSTEES OF
THE LELAND STANFORD JUNIOR UNIVERSITY,
Patent Owner.

Case IPR2017-00013
Patent 7,563,584 B2

Before ERICA A. FRANKLIN, GEORGIANNA W. BRADEN, and
ZHENYU YANG, *Administrative Patent Judges*.

FRANKLIN, *Administrative Patent Judge*.

DECISION
Institution of *Inter Partes* Review
37 C.F.R. § 42.108

I. INTRODUCTION

Fluidigm Corporation (“Petitioner”) filed a Petition requesting an *inter partes* review of claims 1–27 of U.S. Patent No. 7,563,584 B2 (Ex. 1001, “the ’584 patent”). Paper 1 (“Pet.”). The Board of Trustees of the Leland Stanford Junior University (“Patent Owner”) did not file a Preliminary Response to the Petition.¹

We have jurisdiction under 35 U.S.C. § 314, which provides that an *inter partes* review may not be instituted “unless . . . there is a reasonable likelihood that the petitioner would prevail with respect to at least 1 of the claims challenged in the petition.” 35 U.S.C. § 314(a); *see also* 37 C.F.R. § 42.4(a) (delegating authority to the Board). Upon considering the Petition, we determine that Petitioner has shown a reasonable likelihood that it would prevail in showing the unpatentability of claims 1–27. Accordingly, we institute an *inter partes* review of those claims.

A. *Related Proceedings*

Petitioner and Patent Owner affirm that they are not aware of any judicial proceeding involving the ’584 patent. Pet 3, Paper 4, 1.

B. *The ’584 Patent*

The ’584 patent relates to methods for detecting, simultaneously, or sequentially, the activation state of a plurality of activatable proteins in

¹ Although Patent Owner did not file a Preliminary Response, the burden remains on Petitioner to demonstrate unpatentability. *See Dynamic Drinkware, LLC v. Nat’l Graphics, Inc.*, 800 F.3d 1375, 1378 (Fed. Cir. 2015) (citing *Tech. Licensing Corp. v. Videotek, Inc.*, 545 F.3d 1316, 1326–27 (Fed. Cir. 2008)) (discussing the burden of proof in *inter partes* review).

single cells using flow cytometry. Ex. 1001, 4:42–45, 24:55–67. The methods employ activation state-specific antibodies that bind to a specific isoform of activatable protein. *Id.* at 4:61–64. The activation state-specific antibodies may be conjugated to fluorescent labels or fluorescence resonance energy transfer (“FRET”) labels. *Id.* at 4:65–67.

The Specification explains that the disclosed methods allow “rapid detection of heterogeneity in a complex cell population based on protein activation states, and the identification of cellular subsets that exhibit correlated changes in protein activation within the cell population.” *Id.* at 4:30–33.

C. Illustrative Claim

Claim 1 of the ’584 patent is the only independent claim and it is reproduced below:

1. A method of detecting the activation state of at least a first and a second activatable protein in single cells, said method comprising the steps of:
 - a) providing a population of cells comprising said first and said second activatable proteins, wherein said first and second activatable proteins are distinct proteins that each have at least an activated isoform, and a non-activated isoform;
 - b) permeabilizing said population of cells;
 - c) contacting said permeabilized population of cells with at least two distinguishably labeled activation state-specific antibodies,wherein a first of said at least two distinguishably labeled activation state-specific antibodies is specific for said activated isoform of said first activatable protein; and a second of said at least two distinguishably labeled activation state-specific antibodies is specific for said activated isoform of said second activatable protein; and

- d) using flow cytometry to detect binding of said first and said second distinguishably labeled activation state-specific antibodies to their corresponding activated isoform of said first and second activatable proteins in single cells of said population of cells, wherein said binding of said first distinguishably labeled activation state-specific antibody is indicative of the activation state of said first activatable protein, and said binding of said second distinguishably labeled activation state-specific antibody is indicative of the activation state of said second activatable protein.

Ex. 1001, 151:2–30.

D. The Asserted Grounds of Unpatentability

Petitioner challenges the patentability of claims 1–27 of the '584 patent on the following grounds:

Claims	Basis	References
1, 2, 4–6, 10–21, and 23–27	§ 103(a)	Fleisher ²
3, 7–9, and 22	§ 103(a)	Fleisher and Belloc ³
1, 2, 4–6, 10–21, and 23–27	§ 103(a)	Darzynkiewicz ⁴ and Yen ⁵
3, 7–9, and 22	§ 103(a)	Darzynkiewicz, Yen, and Belloc

Petitioner also relies upon the Declaration of Tom Huxford, Ph.D. (Ex. 1002).

² Thomas A. Fleisher et al., *Detection of Intracellular Phosphorylated STAT-1 by Flow Cytometry*, 90 CLINICAL IMMUNOLOGY 425–430 (1999) (Ex. 1004) (“Fleisher”).

³ F. Belloc et al., *Flow Cytometry Detection of Caspase 3 Activation in Preapoptotic Leukemic Cells*, 40 CYTOMETRY 151–160 (2000) (Ex. 1007) (“Belloc”).

⁴ Patent Application Publication No. WO 99/44067 A1 by Zbigniew Darzynkiewicz et al., published Sep. 2, 1999 (Ex. 1005) (“Darzynkiewicz”).

⁵ Andrew Yen et al., *Retinoic Acid Induced Mitogen-activated Protein (MAP)/Extracellular Signal-regulated Kinase (ERK) Kinase-dependent MAP Kinase Activation Needed to Elicit HL-60 Cell Differentiation and Growth Arrest*, 58 CANCER RESEARCH 3163–3172 (1998) (Ex. 1006) (“Yen”).

II. ANALYSIS

A. *Claim Construction*

In an *inter partes* review, the Board interprets claim terms in an unexpired patent according to the broadest reasonable construction in light of the specification of the patent in which they appear. 37 C.F.R. § 42.100(b); *Cuozzo Speed Techs., LLC v. Lee*, 136 S. Ct. 2131, 2142 (2016) (affirming applicability of broadest reasonable construction standard to *inter partes* review proceedings). Under that standard, and absent any special definitions, we give claim terms their ordinary and customary meaning, as would be understood by one of ordinary skill in the art at the time of the invention. *In re Translogic Tech., Inc.*, 504 F.3d 1249, 1257 (Fed. Cir. 2007). Any special definitions for claim terms must be set forth with reasonable clarity, deliberateness, and precision. *In re Paulsen*, 30 F.3d 1475, 1480 (Fed. Cir. 1994).

Petitioner notes that the '584 patent defines certain claim terms, including “activation state-specific antibody.” Pet. 7. According to the Specification, “the term ‘activation state-specific antibody’ or ‘activation state antibody’ or grammatical equivalents thereof, refer to an antibody that specifically binds to a corresponding and specific antigen.” Ex. 1001, 25:21–24. Petitioner recognizes that definition as the broadest reasonable construction of the claim term. Pet. 7. Petitioner also asserts, however, that definition encompasses “virtually any antibody, as all antibodies bind to a specific antigen.” *Id.* For that reason, Petitioner proposes that it may be appropriate to construe the term more narrowly to mean “an antibody that specifically binds to a corresponding and specific isoform of an activatable protein.” *Id.* (citing Ex. 1002 ¶¶ 54–56). Petitioner makes the point that,

based on the disclosure of the Specification, a person of ordinary skill in the art at the time of the invention would consider an “activation state-specific antibody” as referring to an “antibody that specifically binds to a corresponding and specific isoform of an activatable protein.” *Id.* We decline, however, to substitute that construction for the definition expressly provided by the Specification, as it is set forth with reasonable clarity, deliberateness, and precision. *See In re Paulsen*, 30 F.3d at 1480. Moreover, independent claim 1 further describes an “activation state-specific antibody” in a manner that identifies such antibody as “specific for [an] activated isoform of [an] activatable protein.” Ex. 1001, 151:15–16 (Claim 1).

B. Level of Ordinary Skill in the Art

The level of skill in the art is a factual determination that provides a primary guarantee of objectivity in an obviousness analysis. *Al-Site Corp. v. VSI Int’l Inc.*, 174 F.3d 1308, 1324 (Fed. Cir. 1999) (citing *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966); *Ryko Mfg. Co. v. Nu-Star, Inc.*, 950 F.2d 714, 718 (Fed. Cir. 1991)).

Petitioner asserts that the level of skill in the art is high. Pet. 15. According to Petitioner, a person of ordinary skill in the art at the time of the invention would have had “a Ph.D. in the areas of chemistry, biochemistry, cell biology or molecular biology including five or more years of experience in dealing with antibodies, protein labeling, protein interaction, and protein detection.” *Id.* (citing Ex. 1002 ¶¶ 12–13).

At this stage in the proceeding, we determine that Petitioner’s description of the level of ordinary skill in the art to be accurate and supported by the current record. Moreover, we have reviewed Dr.

Huxford's credentials (Ex. 1003) and, at this stage in the proceeding, we consider him to be qualified to provide his opinion on the level of skill and the knowledge of a person of ordinary skill in the art at the time of the invention. We also note that the applied prior art reflects the appropriate level of skill at the time of the claimed invention. *See Okajima v. Bourdeau*, 261 F.3d 1350, 1355 (Fed. Cir. 2001).

C. Obviousness over Fleisher

Petitioner asserts that claims 1, 2, 4–6, 10–21, and 23–27 are unpatentable as obvious over Fleisher. Pet. 15–35.

1. Fleisher

Fleisher is a journal article discussing an investigation of interferon- γ activation of human monocytes using flow cytometry and monoclonal antibodies that distinguish between the native and phosphorylated forms of signal transducer and activator of transcription (1) proteins (“STAT-1”). Ex. 1004, 425. Fleisher explains that its approach “enables rapid and quantitative assessment of STAT-1 phosphorylation on a discrete cell basis and is both more sensitive and less time consuming than immunoblotting.”

Id. In particular, Fleisher describes the method as follows:

Peripheral blood mononuclear cells (PBMC) were . . . prepared . . . in phosphate-buffered saline (PBS) with 2% fetal calf serum and 400 μ l aliquots were cultured without or with interferon- γ (100 or 1000 IU/ml in the standard assay) at 37°C for the times indicated. Following incubation, the cells were either treated with specific antibodies or subjected to fixation and permeabilization before antibody addition . . . 100 μ l of permeabilization medium . . . together with a specific antibody was added to each cell pellet followed by a 30-min incubation at room temperature. The tubes were then washed, incubated with the appropriate second antibody for 30 min at room temperature, and again washed before being resuspended in 200

μl of PBS for flow cytometry. An augmented fixation and permeabilization method involved the addition of 3 ml of cold methanol while vortexing in between the addition of Reagents A and B (3). The tubes were held for 10 min at 4°C, centrifuged, washed in PBS, and resuspended in permeabilization medium (Reagent B) with antibody as described above.

The precultured unmodified PBMC, fixed and permeabilized (F/P) PBMC, and fixed and permeabilized including methanol (F/P + MeOH) PBMC were incubated with 1 μg of murine monoclonal anti-human STAT-1 cytoplasmic terminus (Transduction Laboratories, Lexington, KY), 1 μg of murine IgG2b, 0.1 μg of rabbit anti-human phosphorylated STAT-1 (New England Biolabs, Beverly, MA), or 0.1 μg of rabbit IgG for 30 min as above. The second antibody consisted of either 1 μg of FITC-conjugated F(ab')₂ goat anti-murine IgG (Caltag) or 1 μg of FITC conjugated F(ab')₂ goat anti-rabbit IgG (Caltag) with a 30-min incubation at room temperature as above. Following a final wash step, the cells were resuspended in 200 μl of PBS and analyzed with a flow cytometer.

Id. at 425–426. Fleisher also describes using a FACScan, i.e., a fluorescent activated cell sorting (“FACS”) flow cytometer. *Id.* at 426. According to Fleisher, the disclosed “technique should find applications in the study of multiple phosphorylation-dependent pathways such as those involving other Jak-STAT combinations, IκB, and MAP kinases.” Ex. 1004, 429. Further, Fleisher explains that its approach “should be valuable in studying any activation pathway for which antibody reagents exist that discriminate between a native and an activation modified protein.” *Id.*

2. Analysis

“The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results.” *KSR Int’l Co. v. Teleflex Inc.*, 550 U.S. 398, 416 (2007). “If a person of

ordinary skill can implement a predictable variation, § 103 likely bars its patentability.” *Id.* at 417.

Independent claim 1 recites a method of detecting the activation state of at least a first and a second activatable protein in single cells. Petitioner asserts that a person of ordinary skill in the art would have found the claimed method obvious based on the teachings of Fleisher and the knowledge in the art at the time of the invention. Pet. 32–33. Based on the information presented at this stage of the proceeding, as discussed below, we determine that Petitioner has established sufficiently a reasonable likelihood of prevailing in that regard.

Petitioner asserts that Fleisher teaches a method of detecting the activation state of a first activatable protein, i.e., STAT-1, in single cells comprising: (a) providing a population of cells, PBMC comprising activatable STAT-1 proteins, wherein the protein has at least an activated isoform, and a non-activated isoform; (b) permeabilizing the PBMC; (c) contacting the permeabilized population of cells with a labeled activation state-specific antibody, wherein the antibody is specific for the activated isoform of STAT-1; and (d) using flow cytometry to detect binding of the antibody to its corresponding activated isoform of STAT-1 protein, wherein such binding is indicative of the activation state of the STAT-1. *Id.* at 16–22.

Petitioner acknowledges that Fleisher does not expressly teach using a state-specific antibody to detect the activation state of a second activatable protein. *Id.* at 15. Petitioner asserts, however, that Fleisher explains that its method may apply generally to evaluate other activatable signaling proteins, where activation state-specific antibodies exist. *Id.* at 16 (citing Ex. 1004,

425; Ex. 1002 ¶ 64). Further, Petitioner asserts that the following statement in Fleisher suggests other activatable proteins for evaluation: “This technique should find applications in the study of multiple phosphorylation-dependent pathways such as those involving other Jak-STAT combinations, IκB, and MAP kinases.” *Id.* (quoting Ex. 1004, 429). Based on that statement, Petitioner asserts that Fleisher teaches one of skill in the art that its method may be used to analyze multiple different activatable proteins in a single cell. *Id.* at 16–17. Additionally, Petitioner asserts that a person of skill in the art would have understood that the population of human monocyte cells, i.e., PBMC, prepared and permeabilized in Fleisher’s method, contain MAP kinases, including ERK1/2 and MEK 1/2. *Id.* at 17 (citing Ex. 1002 ¶¶ 70, 73).

Petitioner asserts also that Fleisher’s instruction that its “technique should find application in the study of multiple phosphorylation-dependent pathways,” along with its discussion that the technique may be applied to MAP kinases, would have provided a person of ordinary skill in the art a reason to perform Fleisher’s method using activation state-specific antibodies to detect MAP kinases (ERK1/2 and MEK1/2), in addition to using an activation state-specific antibody to detect the activated STAT-1. Pet. 18 (quoting Ex. 1004, 425 and 429; citing Ex. 1002 ¶¶ 70, 73). Petitioner asserts that Fleisher informed a skilled artisan that such a modification would have only required “the existence of an antibody specific to the activated form of the [additional] proteins of interest.” *Id.* (citing Ex. 1004, 429; Ex. 1002 ¶ 79).

Furthermore, Petitioner's declarant, Dr. Huxford, explains that a person of skill in the art would have known that antibodies specific to the phosphorylated form of ERK1/2 and MEK1/2 were commercially available. Ex. 1002 ¶¶ 22–24, 71, 74. Petitioner and Dr. Huxford assert that one of skill in the art would have understood that those known antibodies could be distinguishably labeled using well known fluorescent labels, such as FITC and PE. Pet. 19–21 (citing Ex. 1002 ¶¶ 74–75). Additionally, Petitioner and Dr. Huxford assert that multi-parametric flow cytometers that could simultaneously distinguish between those fluorescent labels were also well known at the time of the invention. *Id.* at 19 (citing Ex. 1002 ¶¶ 32–34, 72, 74).

Thus, according to Petitioner and Dr. Huxford, it would only require routine optimization to apply Fleisher's teachings to detect the activation state of additional proteins using those known antibodies and known labeling and flow cytometry techniques. Pet. 18–23; Ex. 1002 ¶¶ 66–77. Petitioner and Dr. Huxford explain that a skilled artisan would have understood that doing so would provide “a better understanding of the protein phosphorylation cascade for the MAPK signaling pathway.” Pet. 19–21; Ex. 1002 ¶ 76. Petitioner asserts that goal is consistent with Fleisher's statement describing a benefit of its technique as “permit[ting] dissection of a full range of cellular signaling pathways.” Pet. 20 (quoting Ex. 1004, 425).

Moreover, Petitioner notes that Fleisher explains that its technique is “applicable in any setting where immunoblotting has already been useful to dissect intracellular signaling pathways.” *Id.* at 21 (quoting Ex. 1004, 425). Petitioner asserts that one of skill in the art would have been aware of

“numerous immunoblot assays that detected multiple different intracellular signaling proteins at the same time.” *Id.* Thus, we understand Petitioner explains that Fleisher’s teachings would have provided a skilled artisan with a reasonable expectation of successfully detecting multiple different activatable proteins by applying Fleisher’s method.

On the current record, we discern no deficiency in Petitioner’s characterization of the knowledge in the art at the time of the invention, Fleisher’s teachings, or in Petitioner’s assertions as to the reasonable inferences an ordinary artisan would make from Fleisher. Thus, based on the information presented at this stage of the proceeding, we are satisfied Petitioner has shown sufficiently that there is a reasonable likelihood that it would prevail in showing the unpatentability of independent claim 1 as obvious over Fleisher. We have considered also Petitioner’s arguments and evidence with respect to the challenged dependent claims. *See* Pet. 23–35. Based on the current record, we determine that Petitioner has made a sufficient showing as to those claims, as well. Accordingly, we institute an *inter partes* review of claims 1, 2, 4–6, 10–21, and 23–27 of the ’584 patent as obvious over Fleisher.

D. Obviousness over Fleisher and Belloc

Petitioner asserts that claims 3, 7–9, and 22 are unpatentable as obvious over Fleisher and Belloc. Pet. 35–39.

1. Belloc

Belloc is a journal article describing the use of flow cytometry to detect caspase 3 activation in preapoptotic leukemic cells. Ex. 1007, Background. “Procaspase 3 is a constitutive proenzyme that is activated by cleavage during apoptosis.” *Id.* According to Belloc, “[c]aspase activation

is now well known as a key event in the apoptotic process. In particular, caspase 3 activity is the common effector of most of the apoptotic pathways.” *Id.* at 152. Belloc explains, “[b]ecause active caspase 3 is a common effector in several apoptotic pathways, it may be a good marker to detect (pre-) - apoptotic cells by flow cytometry.” *Id.* at Background. Specifically, Belloc found that “caspase 3 activation can be detected by the anti-cleaved caspase 3 antibodies for the purpose of [flow cytometry] analysis, thus providing an early marker of apoptosis.” *Id.* at 152. According to Belloc, “[t]his labeling can be used in multivariate analysis of bone marrow samples to reveal in vitro apoptosis of leukemic blast cells.” *Id.* Belloc also describes sorting cells by flow cytometry based upon detection of fluorescently labeled antibodies conjugated to proteins of interest, including activated caspase 3. *Id.* at 153, 157, Fig. 4.

2. Analysis

Dependent claims 3, 7–9, and 22 are directed to methods wherein the activatable protein is a caspase, and or the activatable protein is created through protein cleavage. Each of those claims are reproduced below:

3. The method according to claim 1, wherein said first activatable protein is a caspase.
7. The method according to claim 1, wherein said first activatable protein is a first caspase and said second activatable protein is a second caspase.
8. The method according to claim 7, wherein said activated isoform of said first caspase is a cleaved product of a first pro-caspase, and said activated isoform of said second caspase is a cleaved product of a second pro-caspase.

9. The method according to claim 8, wherein said at least two distinguishably labeled activation state-specific antibodies comprise a first distinguishably labeled activation state-specific antibody that is specific for said activated isoform of said first caspase, and a second distinguishably labeled activation state-specific antibody that is specific for said activated isoform of said second caspase.

22. The method according to claim 1, wherein the activated isoform of either the first or second activatable protein is created through protein cleavage.

Petitioner again relies on Fleisher as teaching or suggesting each limitation of independent claim 1. Pet. 35. For dependent claims 3, 7–9, and 22, Petitioner relies also on Belloc for its teaching that those of skill in the art seeking to better understand cell cycle regulation were interested in analyzing the active isoforms of caspases. *Id.* Petitioner supports that contention by referring to Belloc’s teaching that “[c]aspase activation is now well known as a key event in the apoptotic process” and its discovery that “caspase 3 activation can be detected by the anti-cleaved caspase 3 antibodies” and “labeling can be used in multivariate analysis.” *Id.* (quoting Ex. 1007, 152–153).

Petitioner asserts also that Belloc describes concurrently detecting fluorescently labeled antibodies conjugated to multiple proteins of interest, including activated caspase 3, using flow cytometry. *Id.* (citing Ex. 1007, 153, 159). Although Belloc does not specifically address caspase 1, Dr. Huxford explains that both caspase 1 and caspase 3 are involved in the regulation of apoptosis and that other antibodies for those active caspases were known at the time of the invention and used in immunoassays. Ex. 1002 ¶¶ 97–98. Thus, according to Petitioner and Dr. Huxford, a person of

skill in the art would have found it obvious to combine Fleisher and Belloc's teachings to concurrently detect caspases 1 and 3 using activation state-specific antibodies to further understand cell regulation through apoptosis. Pet. 36 (citing Ex. 1002 ¶¶ 99–100).

Petitioner has set forth arguments and evidence with respect to each of the challenged dependent claims. *See* Pet. 36–38 (separately addressing claims 3, and 7–9, and discussing the limitation of claim 22, i.e., creation of an activatable protein through protein cleavage, therein). On the current record, we discern no deficiency in Petitioner's characterization of the knowledge in the art at the time of the invention, the teachings of Fleisher and Belloc, or in Petitioner's assertions as to the reasonable inferences an ordinary artisan would make from those combined teachings. Thus, based on the information presented at this stage of the proceeding, we are satisfied Petitioner has shown sufficiently that there is a reasonable likelihood that it would prevail in showing the unpatentability of claims 3, 7–9, and 22 as obvious over the combined teachings of Fleisher and Belloc. Accordingly, we institute an *inter partes* review of claims 3, 7–9, and 22 as obvious over Fleisher and Belloc.

E. Obviousness over Darzynkiewicz and Yen

Petitioner asserts that claims 1, 2, 4–6, 10–21, and 23–27 are unpatentable as obvious over Darzynkiewicz and Yen. Pet. 39–62.

1. Darzynkiewicz

Darzynkiewicz is directed to methods, reagents and kits that “permit the concurrent and discriminable detection of discrete functional conformations of proteins within a single cell.” Ex. 1005, 7:2–6. The invention focuses on the protein encoded by the retinoblastoma

susceptibility gene (“pRB” or “pRb”) “which plays a pivotal role in the regulation of the cell cycle.” *Id.* at 2:23–26. pRB restrains cell cycle progression in a manner that allows tumor suppression. *Id.* Darzynkiewicz explains that pRB activity is controlled by changes in phosphorylation. *Id.* at 3:4–5. For example, phosphorylated pRB discharges transcription factors, and those factors in turn activate transcription of genes coding for proteins regulating DNA replication and cell proliferation. *Id.* at 3:13–16. The invention provides methods and materials for “the flow cytometric determination of multiple pRB phosphorylation states in individual cells” using anti-pRB antibodies that distinguish the phosphorylation state of pRB and that may be conjugated to fluorophores to allow concurrent detection of such functional conformations of pRB in single cells. *Id.* at 7:6–25.

In addition to comprising contacting a cell with a first and a second antibody specific for two different phosphorylation conformations of pRB, *id.* at 9, Darzynkiewicz explains that the method may further comprise contacting the cell with a third antibody, wherein the third antibody is “specific for a second protein and is distinguishable from each of said first and second antibodies, and then detecting the concurrent binding of each of said antibodies to said cell,” *id.* at 10:8–13. Darzynkiewicz discloses preferred embodiments wherein “the second protein may be a cyclin, a cyclin dependent kinase, or a cyclin dependent kinase inhibitor.” *Id.* at 10:13–16.

Darzynkiewicz teaches that “multiparametric flow cytometric techniques permit more than two antibodies to be discriminably detected in a single assay.” *Id.* at 27:22–24. As an example, Darzynkiewicz explains that its methods may be used, along with such antibodies, “to report,

concurrently with the phosphorylation status of pRB, the concurrent levels of other proteins that participate in the regulation of the cell cycle.” *Id.* at 27:28–33.

2. *Yen*

Yen is a journal article describing a study revealing that retinoic acid (“RA”) “augments MEK-dependent ERK2 activation that is needed for subsequent RB hypophosphorylation, cell differentiation, and G₀ arrest.” Ex. 1006, Abstract. In the study, Western blotting of RB, MAPK, and activated MAPK was performed, wherein cell membranes were “probed with antibodies detecting the phosphorylated and unphosphorylated forms of RB, ERK2, and ERK1.” *Id.* at 3165.

3. *Analysis*

As previously discussed, independent claim 1 recites a method of detecting the activation state of at least a first and a second activatable protein in single cells. Petitioner asserts that a person of ordinary skill in the art would have found the claimed method obvious based on the teachings of Darzynkiewicz and *Yen*, along with the knowledge in the art at the time of the invention. Pet. 39. Based on the information presented at this stage of the proceeding, as discussed below, we determine that Petitioner has established sufficiently a reasonable likelihood of prevailing in that regard.

Petitioner asserts that the combined teachings of Darzynkiewicz and *Yen* teach or suggest each limitation of independent claim 1. Pet. 41–50. In particular, Petitioner asserts that Darzynkiewicz teaches a method of detecting two different proteins in a single cell. *Id.* at 41 (citing Ex. 1005, 10:13–16). Petitioner recognizes that Darzynkiewicz does not describe the second protein to be an activated isoform. *Id.* at 39. Petitioner, however,

contends that a person of skill in the art would understand from Darzynkiewicz that its method of using distinguishable antibodies inclusively applies to using activation state-specific antibodies specific for an activated isoform of the second protein. *Id.* (citing Ex. 1002 ¶¶ 16–17). Further, according to Petitioner and Dr. Huxford, doing so would not require any change to Darzynkiewicz’s approach. *Id.* at 39–40 (citing Ex. 1002 ¶ 17).

Petitioner asserts also that Darzynkiewicz applies its method to the human promyelocytic leukemic cell line, HL-60. *Id.* at 42; Ex. 1005, 24:15–16. Petitioner refers also to the teaching in Darzynkiewicz that, in its method, the “[c]ells may, for example, be simultaneously fixed and permeabilized in HBSS (HEPES buffered saline)” or “permeabilized using commercially available reagents, such as FACS™ Permeabilization Solution.” Ex. 1005, 25:16–21; Pet. 43;. According to Petitioner and Dr. Huxford, a person of skill in the art would have understood that the HL-60 cell line contains many different activatable and distinct proteins, including retinoblastoma, Ras, ERK1/2 and MEK1/2. Pet. 42 (citing Ex. 1002 ¶¶ 103–104). In further support of that contention, Petitioner refers to Yen’s discussion relating to detecting retinoblastoma in addition to activated ERK1/2, using an activation state-specific antibody specific for activated ERK1/2, to analyze the effect of those proteins on cell cycle regulation. *Id.* at 40, 42, 44 (citing Ex. 1006, 3163, 3165).

Additionally, Petitioner asserts that Darzynkiewicz explains that after permeabilizing the population of cells, those “cells are labeled with antibodies conjugated to flow cytometrically detectable fluorophores.” Pet. 43 (quoting Ex. 1005, 25:29–31). Furthermore, Petitioner refers to the

teaching in Darzynkiewicz that it is well known in the art that “there are a number of two-fluorophore combinations that permit concurrent and discriminable detection,” such as FITC and PE-CY5. *Id.* at 43–44 (citing Ex. 1005, 26:3–24). Petitioner asserts also that Darzynkiewicz describes using “multiparametric flow cytometric techniques [to] permit more than two antibodies to be discriminably detected in a single assay.” *Id.* at 41 (quoting Ex. 1005, 27:22–24). Petitioner refers also to a statement in Darzynkiewicz that “these further antibodies may, for example, be used to report, concurrently with the phosphorylation status of pRB, the concurrent levels of other proteins that participate in the regulation of the cell cycle.” *Id.* (emphasis omitted) (quoting Ex. 1005, 27:29–33).

According to Petitioner, the combined teachings of Darzynkiewicz and Yen would have motivated a person of ordinary skill in the art to analyze the effects of cell cycle regulation by concurrently analyzing the activated state of retinoblastoma protein, ERK1/2, MEK1/2, or RAS using the multiparametric flow cytometric method taught by Darzynkiewicz. *Id.*

On the current record, we discern no deficiency in Petitioner’s characterization of the knowledge in the art at the time of the invention, the teachings of Darzynkiewicz and Yen, or in Petitioner’s assertions as to the reasonable inferences an ordinary artisan would draw from those combined teachings. Thus, based on the information presented at this stage of the proceeding, we are satisfied Petitioner has shown sufficiently that there is a reasonable likelihood that it would prevail in showing the unpatentability of independent claim 1 as obvious over the combined teachings of Darzynkiewicz and Yen. We have considered also Petitioner’s arguments and evidence with respect to the challenged dependent claims. *See* Pet. 50–

62. Based on the current record, we determine that Petitioner has made a sufficient showing as to those claims, as well. Accordingly, we institute an *inter partes* review of claims 1, 2, 4–6, 10–21, and 23–27 of the '584 patent as obvious over Darzynkiewicz and Yen.

F. Obviousness over Darzynkiewicz, Yen, and Belloc

Petitioner asserts that claims 3, 7–9, 22 are unpatentable as obvious over Darzynkiewicz, Yen, and Belloc. Pet. 62–65. We have described the teachings of Darzynkiewicz, Yen, and Belloc in the preceding discussions. As also previously discussed, dependent claims 3, 7–9, and 22 are directed to methods wherein the activatable protein is a caspase, and or the activatable protein is created through protein cleavage.

Petitioner again relies on Darzynkiewicz and Yen as teaching or suggesting each limitation of independent claim 1. Pet. 62. For dependent claims 3, 7–9, and 22, Petitioner relies also on Belloc for its teaching that those of skill in the art seeking to better understand cell cycle regulation were interested in analyzing the active isoforms of caspases. *Id.* Petitioner supports that contention in the same manner discussed above, regarding the challenge of these claims over Fleisher and Belloc.

Petitioner has set forth arguments and evidence with respect to each of the challenged dependent claims. *See* Pet. 62–65 (separately addressing claims 3, and 7–9, and discussing the limitation of claim 22, i.e., creation of an activatable protein through protein cleavage, therein). On the current record, we discern no deficiency in Petitioner's characterization of the knowledge in the art at the time of the invention, the teachings of Darzynkiewicz, Yen, and Belloc, or in Petitioner's assertions as to the reasonable inferences an ordinary artisan would make from those combined

teachings. Thus, based on the information presented at this stage of the proceeding, we are satisfied Petitioner has shown sufficiently that there is a reasonable likelihood that it would prevail in showing the unpatentability of claims 3, 7–9, and 22 as obvious over the combined teachings of Darzynkiewicz, Yen, and Belloc. Accordingly, we institute an *inter partes* review of claims 3, 7–9, and 22 as obvious over Darzynkiewicz, Yen, and Belloc.

III. CONCLUSION

For the foregoing reasons, we determine that the information presented in the Petition establishes that there is a reasonable likelihood that Petitioner would prevail in showing that claims 1–27 of the '584 patent are unpatentable.

At this stage of the proceeding, the Board has not made a final determination as to the patentability of any challenged claim.

ORDER

In consideration of the foregoing, it is hereby:

ORDERED that pursuant to 35 U.S.C. § 314(a), an *inter partes* review is instituted as to claims 1–27 of the '584 patent on the following grounds of unpatentability:

A. Claims 1, 2, 4–6, 10–21, and 23–27 under 35 U.S.C. § 103(a) as obvious over Fleisher;

B. Claims 3, 7–9, and 22 under 35 U.S.C. § 103(a) as obvious over Fleisher and Belloc;

C. Claims 1, 2, 4–6, 10–21, and 23–27 under 35 U.S.C. § 103(a) as obvious over Darzynkiewicz and Yen; and

D. Claims 3, 7–9, and 22 under 35 U.S.C. § 103(a) as obvious over Darzynkiewicz, Yen, and Belloc; and

FURTHER ORDERED that pursuant to 35 U.S.C. § 314(c) and 37 C.F.R. § 42.4, notice is hereby given of the institution of a trial commencing on the entry date of this Decision.

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