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BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte JUAN BALLESTEROS, TERESA BENNETT, DANIEL PRIMO, ALBERTO ORFAO, COYT JACKSON, SANTIAGO LAGO, MARIA MATOSES, LILIA SUAREZ, SANDRA SAPIA, ANDREW BOSANQUET, JULIAN CORROCHATEGUI, CONSUELO TUDELA, PILAR HERNANDEZ, and LUIS IGNACIO CAVEDA[1]

Appeal 2019-005710
Application 15/460,872
Technology Center 1600

Before ERIC B. GRIMES, ULRIKE W. JENKS, and JOHN G. NEW, *Administrative Patent Judges.*

GRIMES, *Administrative Patent Judge.*

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134(a) involving claims to a method of determining the responsiveness of cells to drugs, which have been rejected as indefinite and obvious. We have jurisdiction under 35 U.S.C. § 6(b). We AFFIRM.

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[1] Appellant identifies the real party in interest as Vivia Biotech, S.L. Appeal Br. 3. We use the word Appellant to refer to "applicant" as defined in 37 C.F.R. § 1.42(a).

STATEMENT OF THE CASE

The Specification describes "a cell-based screening platform that incorporates both automated sample preparation and automated evaluation by flow cytometry that is useful as a personalized medicine test because of its rapid data acquisition, analysis, and reporting of results." Spec. ¶ 2.

Claims 56–65 and 67–71 are on appeal. Claim 56, reproduced below, is illustrative:

> 56. A method for analyzing a patient's cellular responsiveness to drugs and for identifying a patient's resistance to a drug composition, comprising:
>
> a. Obtaining a sample of whole blood, whole peripheral blood or whole bone marrow that has been withdrawn from a patient, having a hematological neoplasm;
>
> b. dividing the whole sample into at least 35 aliquots;
>
> c. distributing each aliquot in a well of a plate;
>
> d. combining each of the at least 35 aliquots with the drug composition in the well and incubating said drug composition with the aliquot for more than 4 hours; and
>
> e. counting cells in each of the at least 35 aliquots in an automated flow cytometry platform comprising automated sample preparation and automated input to a flow cytometer, thereby providing counts of cells, wherein the counts of cells that are no longer alive relative to a control aliquot without the drug composition provides a percentage of cancer cell depletion.

The claims stand rejected as follows:

Claims 56–65 and 67–71 under 35 U.S.C. § 112, second paragraph, as indefinite (Ans. 3);

Claims 56–65 and 67–71 under 35 U.S.C. § 103(a) as obvious based on Mitsuhashi I,[2] Mitsuhashi II,[3] Edwards,[4] and Bargou[5] (Ans. 3–4); and

Claims 56–65 and 67–71 under 35 U.S.C. § 103(a) as obvious based on Mitsuhashi I, Mitsuhashi II, Edwards, and Mølhøj[6] (Ans. 8).

OPINION

Definiteness

Claims 56–65 and 67–71 stand rejected under 35 U.S.C. § 112, second paragraph, on the basis that "[t]here is insufficient antecedent basis for 'the counts of cells that are no longer alive.'" Ans. 3. The Examiner notes, however, that "the following amendment to claim 56 would obviate this rejection: '~~**the**~~ counts of cells that are no longer alive'." *Id*.

"Appellant . . . would be amenable to an Examiner's amendment consistent with the Examiner's suggestion should prosecution be reopened or the case is otherwise allowed." Appeal Br. 9.

Because Appellant does not dispute the merits of the rejection under 35 U.S.C. § 112, second paragraph, we affirm it.

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[2] *Ex Vivo Simulation of the Action of Antileukemia Drugs by Measuring Apoptosis-Related mRNA in Blood*, Clinical Chemistry 54:673−681 (2008).
[3] *Ex Vivo Induction of mRNA in Human Whole Blood as a New Platform of Drug and Dietary Supplement Development*, Pharmaceutical Research 25:1116−1124 (2008).
[4] *High-Throughput Cytotoxicity Screening by Propidium Iodide Staining*, Current Protocols in Cytometry 9.24.1−9.24.11 (2007).
[5] *Tumor Regression in Cancer Patients by Very Low Doses of a T Cell-Engaging Antibody*, Science 231:974–977 (2008).
[6] *CD19-/CD3-bispecific antibody of the BiTE class is far superior to tandem diabody with respect to redirected tumor cell lysis*, Molecular Immunology 44:1935−1943 (2007).

Obviousness

Claims 56–65 and 67–71 stand rejected as obvious based on Mitsuhashi I, Mitsuhashi II, Edwards, and either Bargou or Mølhøj. Appellant relies on the same arguments with respect to both rejections. Appeal Br. 19. We will therefore address them together.

> The Examiner finds that Mitsuhashi I teaches a method comprising
>
> providing a whole sample (whole blood) obtained from the patient that includes neoplastic cells; incubating numerous aliquots of the whole sample with a different drug or combination of drugs . . . "overnight"; analyzing each aliquot of the whole sample with methods including using flow cytometer (FACS) and FITC-labeled Annexin V to count live cells vs those cells becoming apoptotic in aliquots relative to control aliquots . . . ; and generating a report of results of a plurality of drugs or drug combinations from the analysis step indicating the degree of apoptosis or cell depletion of each treated aliquot.

Ans. 4. The Examiner cites Mitsuhashi II for its disclosure of the advantages of whole blood assay systems, like those in Mitsuhashi I, over systems using cells in culture. *Id*. at 5.

The Examiner finds that Mitsuhashi I does not teach using at least thirty-five aliquots from a single patient, *id*., but that Edwards teaches

> a high-throughput automated method of screening cytotoxic activity of compounds comprising obtaining a sample of cancer cells, dividing the sample into 384 aliquots, distributing each aliquot in a well of a plate, combining each of the aliquots with a drug in the well, incubating the drug composition with the aliquot for at least 24 hours, and counting cells in each aliquot in an automated flow cytometry platform comprising automated sample preparation and automated input to a flow cytometer, thereby providing counts of cells, wherein counts of cells that are no longer alive relative to a control aliquot without the drug composition provides a percentage of cancer cell depletion.

Id. at 6. The Examiner cites both Bargou and Mølhøj for their disclosure of the elected species of drug (blinatumomab) and assays to determine its therapeutic effect based on cell lysis. *Id*. at 6, 10.

> The Examiner concludes that it would have been obvious
>
> to predict responsiveness of leukemia and lymphoma patients to cancer treatments by performing the method of Mitsuhashi I using numerous (including 100+) aliquots of whole blood wherein each aliquot is in a well . . . using a high-throughput automated flow cytometry platform comprising automated sample preparation and automated input to a flow cytometer wherein each aliquot is treated with various therapeutics.

Id. at 6–7. The Examiner also concludes that it would have been obvious to measure apoptosis and/or cell lysis "because therapeutics are known to function by inducing apoptosis (as demonstrated by Mitsuhashi I)" and both Bargou and Mølhøj teach measuring drug effectiveness based on cell depletion or lysis. *Id*. at 7, 11. Finally, the Examiner concludes that it would have been obvious to use Edwards' automated flow cytometry method because "a high-throughput automated method that has the benefit of overcoming time delay issues associated with conventional flow cytometry handling." *Id*. at 7.

We agree with the Examiner that the method of claim 56 would have been obvious to a person of ordinary skill in the art based on the cited references. Mitsuhashi I discloses a method for predicting the response of individual cancer patients to particular drugs. Mitsuhashi I 673, right col. Mitsuhashi I discloses an assay for leucocyte apoptosis based on mRNA expression levels of p21 and PUMA. *Id*. at 674, left col.

Mitsuhashi I states that samples of whole blood were obtained from "patients with various blood malignancies." *Id*. Mitsuhashi I also states that

blood was drawn from eighteen patients with acute myelogenous leukemia (AML) and "stimulated with the appropriate drugs." *Id*. at 674, right col. Mitsuhashi I teaches that 70 µL aliquots of blood samples were combined with specific drugs, at specific concentrations. *Id*. (under the heading "BLOOD STIMULATION").

Mitsuhashi I describes mRNA quantification for p21 and PUMA. *Id*. at 674–675. Mitsuhashi I also states that they carried out annexin V analysis by "incubat[ing] 70 µL samples of blood from 5 AML patients overnight with the drug," then lysing erythrocytes, and incubating the resulting leukocyte suspension with fluorescein isothiocyanate-labeled annexin V and propidium iodide. *Id*. at 675, left col. Mitsuhashi I "then analyzed annexin V binding via flow cytometry." *Id*. at 675, right col.

Mitsuhashi I compared the results of the mRNA assay with "conventional apoptosis assays," including "the annexin V fluorescence-activated cell sorting (FACS) analysis." *Id*. (under the heading "mRNA ASSAY VERSUS CONVENTIONAL APOPTOSIS ASSAYS"). Mitsuhashi I concludes that "[t]he ex vivo mRNA analyses yielded more positive results than the biological assays" (including the annexin V FACS assay), which "indicates that the mRNA assay may have better analytical sensitivity than these [] conventional assays, or that it may produce more false-positive reactions." *Id*. at 678, right col.

Thus, Mitsuhashi I teaches a method for analyzing a patient's response to a drug comprising obtaining a sample of whole blood from a patient having a hematological neoplasm; dividing the sample into aliquots, combining the aliquots with a drug, and incubating overnight; then using the

conventional annexin V FACS (i.e., flow cytometry) assay to count apoptotic cells as a measure of cell death. *Cf.* Spec. ¶ 91: "This system can determine the ex vivo therapeutic index by measuring the ability of a drug composition to induce apoptosis. . . . In a specific embodiment, the method uses Annexin V coupled to Fluorescein Isothiocyanate (FITC) to detect . . . apoptotic cells."

Mitsuhashi I does not disclose (a) dividing a sample into at least 35 aliquots, (b) distributing aliquots to wells of a plate, or (c) carrying out automated flow cytometry comprising automated sample preparation and automated input to a flow cytometer, as recited in claim 56.

However, Edwards describes "a system for the automated high-throughput analysis of cell cytotoxicity in 96-well and 384-well microplates." Edwards 9.24.1. Edwards states that "[a]utomated handling for flow cytometry in sample-tube carousels has recently evolved to microplate formats." *Id*. Edwards provides an example of cytotoxicity screening in 384-well format with automated liquid handling. *Id*. at 9.24.1–9.24.5. Edwards states that "high-throughput automation platforms capable of liquid handling, plate movement, shaking devices, and temperature control are now available in a variety of configurations. Systems capable of dispensing nanoliter volumes into a variety of plate formats have substantially reduced the requirement for expensive or low-abundance reagents and assay setup time." *Id*. at 9.24.7.

Bargou reports that "a bispecific antibody construct called blinatumomab . . . has the potential to engage all cytotoxic T cells in patients for lysis of cancer cells. Doses as low as 0.005 milligrams per square meter

per day in non-Hodgkin's lymphoma patients led to an elimination of target cells in blood." Bargou 974, abstract. "The decline of $CD19^+$ cells [in a patient] was accompanied by expression of apoptosis marker annexin V . . . , indicating lysis . . . of target cells." *Id*. at 975, left col.

Based on the teachings, it would have been obvious to combine the conventional annexin V FACS assay of Mitsuhashi I with Edward's system for automated high-throughput analysis of cell cytotoxicity in 96- or 384-well microplates, because Edwards teaches that the automated platforms capable of liquid handling, etc. substantially reduce the requirement for expensive or low-abundance reagents and reduce assay setup time, thus making the manual process of Mitsuhashi I more efficient. It would also have been obvious to include Bargou's blinatumomab (the elected species of drug) in such a cytotoxicity assay, because Bargou teaches that it has the potential to engage cytotoxic T cells in patients, causing lysis of cancer cells; thus, a skilled artisan would have recognized that it is a suitable candidate for cytotoxicity testing.

Appellant argues that, although Mitsuhashi I used flow cytometry to measure annexin V as an apoptosis marker, "the traditional apoptosis marker analyses are being used to merely prove the efficacy of Mitsuhashi I's new mRNA test." Appeal Br. 13. Appellant argues that

> the results in Mitsuhashi I relating to the Annexin V analysis, i.e., the apoptosis marker, are qualitative to the reader. There is no way to identify which drugs, drug concentrations or combinations of drugs are associated with which data point in Figure 1. The Annexin V results were provided merely to verify if the mRNA experiments proposed by Mitsuhashi I are a viable candidate platform for drug-sensitivity tests in leukemia.

Id. at 15.

Similarly, Appellant argues that

> [i]f we were to combine Edwards with Mitsuhashi I, as proposed by the Examiner herein, all that would happen is that the Annexin V analysis in Mitsuhashi I would be automated. . . . Using an automated flow cytometry system such as that from Edwards in the Mitsuhashi I Annexin V analysis (the only place it would be used) just makes the acquisition of the data for the FACS analysis more rapid.

Id. at 17–18.

These argument are unpersuasive because, as the Examiner has noted, "knowledge of which drugs, drug concentrations, or combinations of drugs are associated with which data point in Figure 1 is not required to perform the method rendered obvious by the combination of cited references." Ans. 14. That is, Mitsuhashi I is cited as evidence that it would have been obvious to use the conventional annexin V FACS (flow cytometry) assay to determine whether a drug was effective to cause apoptosis and cell death in cancer cells from a patient, not as evidence that a particular drug or drug combination was shown to be effective in the samples tested by Mitsuhashi I. Appellant's argument does not show any error in the rejection on appeal.

Appellant also argues that

> Mitsuhashi I reported that the positive results **may be false-positives for the mRNA results**. . . .
>
> Alternatively, Mitsuhashi I disclosed that ex vivo mRNA analysis yielded more positive results than . . . annexin V analys[i]s. . . . [I]n this scenario, there would be no reasonable expectation of success to use the annexin V analysis to analyze a patient's cellular responsiveness to drugs and for identifying a patient's resistance to a drug composition, as claimed by Appellant herein, because the results may be unacceptable because of a lack of sensitivity. Why run hundreds or thousands of individual tests to try to individualize the treatment for a

> person if the results are only as good as the less sensitive detection method?

Appeal Br. 16–17.

This argument is also unpersuasive. As Appellant points out, Mitsuhashi I found that its mRNA assay yielded more positive results than the conventional annexin V FACS assay. Mitsuhashi I 678, right col. Mitsuhashi I states that "[t]his finding indicates that the mRNA assay may have better analytical sensitivity than these 2 conventional assays, or that it may produce more false-positive reactions." *Id*. The uncertainty expressed by Mitsuhashi I regarding whether the mRNA test was actually more sensitive provides sufficient reason to use the conventional (art-accepted) annexin V assay to detect apoptosis, in order to minimize the number of false positive results. Appellant's argument that the annexin V FACS analysis would not provide a reasonable expectation of success is unpersuasive because Mitsuhashi I characterizes it as a "conventional" assay for apoptosis. Mitsuhashi I 678, right col. The assay's conventional nature indicates that it was accepted as sufficiently accurate by those skilled in the art.

For the reasons discussed above, we affirm the rejection of claim 56 under 35 U.S.C. § 103(a) based on Mitsuhashi I, Mitsuhashi II, Edwards, and Bargou. Claims 57–65 and 67–71 fall with claim 56 because they were not argued separately. 37 C.F.R. § 41.37(c)(1)(iv).

Appellant relies on the same arguments with regard to the rejection of claims 56–65 and 67–71 under 35 U.S.C. § 103(a) based on Mitsuhashi I, Mitsuhashi II, Edwards, and Mølhøj. Appeal Br. 19. We therefore affirm that rejection as well.

DECISION SUMMARY

In summary:

Claims Rejected	35 U.S.C. §	Reference(s)/Basis	Affirmed	Reversed
56–65, 67–71	112, second paragraph	Indefiniteness	56–65, 67–71	
56–65, 67–71	103(a)	Mitsuhashi I, Mitsuhashi II, Edwards, Bargou	56–65, 67–71	
56–65, 67–71	103(a)	Mitsuhashi I, Mitsuhashi II, Edwards, Mølhøj	56–65, 67–71	
Overall Outcome			56–65, 67–71	

TIME PERIOD FOR RESPONSE

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a). *See* 37 C.F.R. § 1.136(a)(1)(iv).

AFFIRMED