

invested into validating and optimizing the ability to count absolute cell numbers using flow cytometry.

In order to determine selectivity of a test compound to affect one cell type over another in a complex mixture of cells, the person skilled in the art would consequently measure the EC50 of the test compound towards both cell populations, take the ratio of the the EC50 values or the difference of the log EC50 values as the measure of selectivity (c.f., definition of therapeutic index used by FDA).

However, this approach suffers from major limitations: Absolute cell numbers are inherently difficult to measure using flow cytometry and also other single cell analysis techniques. Absolute cell numbers seeded into assay plates may vary significantly in particular when automated cell dispensing machinery is used. During subsequent staining and washing steps, cells may be lost differentially from different assay wells. Further, absolute cell quantification typically needs to be benchmarked against bead standards which introduces another source of error and represents additional effort. Finally, determination of selectivity will require the measurement of test compound effect at different test compound concentrations which vastly increases the sample requirement and assay time.

In view of the foregoing, there is a need in the art for methods for determining selectivity of chemical compounds in complex cellular mixtures.

The technical problem to be solved by the present invention is thus the provision of improved methods for determining selectivity of one or more test compound(s) and related methods based on the improved determined selectivity.

Summary

In one aspect, the present disclosure refers to a method for determining the selectivity of a test compound, the method comprising the steps: (a) providing a sample

comprising at least two distinguishable sub-populations of cells in a total population of cells; (b) dividing the sample into at least two parts; (c) incubating at least one part obtained in step (b) in the absence of a test compound and at least one part obtained in step (b) in the presence of a test compound; (d) determining the number of cells in one of the at least two sub-populations that exhibit a distinguishable phenotype, relative to the number of cells in the total population of cells that exhibit the same phenotype in (i) the at least one part incubated in the presence of the test compound and (ii) in the at least one part incubated in the absence of the test compound; and (e) determining selectivity of the test compound to induce the phenotype referred to in (d) in the one sub population referred to in step (d) over all other subpopulations by dividing (i) through (ii) wherein the test compound selectively induces the phenotype referred to in (d) if (i) divided through (ii) is greater than 1, preferably greater than 1.05, 1.1, 1.5, 2, 3 most preferably 5, and selectively inhibits or reduces the phenotype referred to in (d) if it is less than 1, preferably less than 0.95, 0.9, 0.7, 0.5, 0.3, most preferably less than 0.2.

In another aspect, the present disclosure refers to a method for determining whether a subject suffering from cancer will respond or is responsive to treatment with a test compound, the method comprising the steps: (a) providing a sample obtained from the subject comprising at least two sub-populations of cells in a total population cells, wherein at least one sub-population corresponds to cancerous cells and at least one sub-population corresponds to non-cancerous cells; (b) dividing the sample into at least two parts; (c) incubating at least one part obtained in step (b) in the absence of a test compound and at least one part in the presence of a test compound; (d) determining the number of viable cells in at least one of the sub-population corresponding to cancer cells relative to the number of viable cells in the total population of cells in (i) the at least one part incubated in the presence of the test compound and (ii) the at least one part incubated in the absence of the test compound; and (e) determining whether the subject will respond or is responsive to treatment with the test compound by dividing (i) through (ii), wherein the subject will respond or is responsive to treatment if the resulting value is less than 1, preferably less than 0.95, 0.9, 0.8, 0.6, 0.4 most preferably less than 0.2.

Description

The invention, in a first embodiment, thus relates to a method for determining the selectivity of a test compound, the method comprising the steps:

- (a) providing a sample comprising at least two distinguishable sub-populations of cells in a total population of cells;
- (b) dividing the sample into at least two parts;