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(57) Настоящее изобретение относится к штамму *Bacteroides thetaiotaomicron* его производным, а также к использованию указанного или производных штаммов в лечении воспалительных, аутоиммунных и аллергических заболеваний. Изобретение также относится к фармацевтическим композициям, питательным добавкам, кормовым продуктам, пищевым продуктам, биологически активным пищевым добавкам и пищевым добавкам, содержащим указанный штамм или его производные.

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## ОПИСАНИЕ ИЗОБРЕТЕНИЯ

2420-543205ЕА/022

### ИММУНОМОДУЛЯЦИЯ

ОБЛАСТЬ ТЕХНИКИ, К КОТОРОЙ ОТНОСИТСЯ ИЗОБРЕТЕНИЕ

Настоящее изобретение относится к микроорганизмам, которые способны положительно модулировать воспалительные заболевания и которые могут быть использованы в терапии или профилактической медицине.

### ПРЕДПОСЫЛКИ СОЗДАНИЯ ИЗОБРЕТЕНИЯ

Бактерии *Bacteroides thetaiotaomicron* оказывают сильное противовоспалительное действие *in vitro* и *in vivo* (Kelly *et al.* Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol. 2004 Jan; 5(1): 104-12). Они модулируют молекулярные сигнальные пути NF-κB (Kelly *et al.*, Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol. 2004 Jan; 5(1): 104-12). В частности, они препятствуют связыванию активного компонента (RelA) NF-κB с ключевыми генами в ядре, тем самым предотвращая активацию провоспалительных путей (Kelly *et al.*, выше, 2004). Полный геном *B. thetaiotaomicron* был секвенирован и аннотирован Gordon Group (Washington University School of Medicine, USA) в 2003 г. [Xu *et al.*, A genomic view of the human-*Bacteroides thetaiotaomicron* symbiosis. Science. 2003 Mar 28; 299(5615): 2074-6].

### СУЩНОСТЬ ИЗОБРЕТЕНИЯ

Настоящее изобретение основано на открытии штамма *Bacteroides thetaiotaomicron* (BT), который обладает удивительной эффективностью против воспалительных заболеваний. Соответственно, штамм BT является полезным в качестве терапевтического или профилактического средства против воспалительных заболеваний и/или аутоиммунных заболеваний, и/или аллергических заболеваний.

Первый аспект изобретения относится к бактерии *Bacteroides thetaiotaomicron*, депонированной под регистрационным номером

NCIMB 42341, или ее производному.

Второй аспект изобретения относится к питательной добавке, содержащей бактерию *Bacteroides thetaiotaomicron*, описанную в пункте 1, и приемлемый для питания эксципиент, носитель или разбавитель.

Третий аспект изобретения относится к кормовому продукту, пищевому продукту, биологически активной пищевой добавке или пищевой добавке, содержащим бактерию *Bacteroides thetaiotaomicron*, описанную в пункте 1.

Четвертый аспект изобретения относится к бактерии *Bacteroides thetaiotaomicron*, описанной в пункте 1, композиции, описанной в пункте 2 или 3, фармацевтической композиции, описанной в пункте 4 или 5, питательной добавке, описанной в пункте 6 или 7, или кормовому продукту, пищевому продукту, биологически активной пищевой добавке или пищевой добавке, описанным в пункте 8 или 9, для применения с целью модуляции воспаления ткани или органа у субъекта.

Пятый аспект изобретения относится к бактерии *Bacteroides thetaiotaomicron*, описанной в пункте 1, композиции, описанной в пункте 2 или 3, фармацевтической композиции, описанной в пункте 4 или 5, питательной добавке, описанной в пункте 6 или 7, или кормовому продукту, пищевому продукту, биологически активной пищевой добавке или пищевой добавке, описанным в пункте 8 или 9, для применения в лечении и/или предотвращении заболевания у субъекта; при этом указанное заболевание представляет собой воспалительное заболевание и/или аутоиммунное заболевание.

Шестой аспект изобретения относится к бактерии *Bacteroides thetaiotaomicron*, описанной в пункте 1, композиции, описанной в пункте 2 или 3, фармацевтической композиции, описанной в пункте 4 или 5, питательной добавке, описанной в пункте 6 или 7, или кормовому продукту, пищевому продукту, биологически активной пищевой добавке или пищевой добавке, описанным в пункте 8 или 9, для применения с целью уменьшения разрыва толстого кишечника у субъекта, предпочтительно, указанный субъект имеет IBD.

Седьмой аспект изобретения относится к бактерии *Bacteroides thetaiotaomicron*, описанной в пункте 1, композиции, описанной в

пункте 2 или 3, фармацевтической композиции, описанной в пункте 4 или 5, питательной добавке, описанной в пункте 6 или 7, или кормовому продукту, пищевому продукту, биологически активной пищевой добавке или пищевой добавке, описанным в пункте 8 или 9, для применения с целью уменьшения экспрессии одного или более провоспалительных генов в клетке или клетках у субъекта.

Восьмой аспект изобретения относится к бактерии *Bacteroides thetaiotaomicron*, описанной в пункте 1, композиции, описанной в пункте 2 или 3, фармацевтической композиции, описанной в пункте 4 или 5, питательной добавке, описанной в пункте 6 или 7, или кормовому продукту, пищевому продукту, биологически активной пищевой добавке или пищевой добавке, описанным в пункте 8 или 9, для применения с целью увеличения процентного содержания регуляторных Т-клеток (Treg) в пищеварительном тракте или секции пищеварительного тракта.

Девятый аспект изобретения относится к способу получения фармацевтической композиции по пункту 4 или 5, включающему смешивание указанной бактерии *Bacteroides thetaiotaomicron* с фармацевтически приемлемым эксципиентом, носителем или разбавителем, при этом указанная бактерия *Bacteroides thetaiotaomicron*, необязательно, инкапсулируется во время указанного процесса.

Десятый аспект изобретения относится к способу модуляции воспаления ткани или органа у субъекта, включающему введение субъекту бактерии *Bacteroides thetaiotaomicron*, описанной в пункте 1, композиции, описанной в пункте 2 или 3, фармацевтической композиции, описанной в пункте 4 или 5, питательной добавки, описанной в пункте 6 или 7, или кормового продукта, пищевого продукта, биологически активной пищевой добавки или пищевой добавки, описанных в пункте 8 или 9.

Одиннадцатый аспект изобретения относится к способу лечения и/или предотвращения воспалительного заболевания и/или аутоиммунного заболевания у субъекта, включающему введение субъекту бактерии *Bacteroides thetaiotaomicron*, описанной в пункте 1, композиции, описанной в пункте 2 или 3, фармацевтической композиции, описанной в пункте 4 или 5,



питательной добавки, описанной в пункте 6 или 7, или кормового продукта, пищевого продукта, биологически активной пищевой добавки или пищевой добавки, описанных в пункте 8 или 9.

Двенадцатый аспект изобретения относится к способу уменьшения разрыва толстого кишечника у субъекта, включающему введение субъекту бактерии *Bacteroides thetaiotaomicron*, описанной в пункте 1, композиции, описанной в пункте 2 или 3, фармацевтической композиции, описанной в пункте 4 или 5, питательной добавки, описанной в пункте 6 или 7, или кормового продукта, пищевого продукта, биологически активной пищевой добавки или пищевой добавки, описанных в пункте 8 или 9, предпочтительно, в случае, когда субъект имеет IBD.

Тринадцатый аспект изобретения относится к способу уменьшения экспрессии одного или более провоспалительных генов в клетке или клетках у субъекта, включающему введение субъекту бактерии *Bacteroides thetaiotaomicron*, описанной в пункте 1, композиции, описанной в пункте 2 или 3, фармацевтической композиции, описанной в пункте 4 или 5, питательной добавки, описанной в пункте 6 или 7, или кормового продукта, пищевого продукта, биологически активной пищевой добавки или пищевой добавки, описанных в пункте 8 или 9.

Четырнадцатый аспект изобретения относится к способу увеличения процентного содержания регуляторных Т-клеток (Treg) в пищеварительном тракте или секции пищеварительного тракта, включающему введение субъекту бактерии *Bacteroides thetaiotaomicron*, описанной в пункте 1, композиции, описанной в пункте 2 или 3, фармацевтической композиции, описанной в пункте 4 или 5, питательной добавки, описанной в пункте 6 или 7, или кормового продукта, пищевого продукта, биологически активной пищевой добавки или пищевой добавки, описанных в пункте 8 или 9.

Пятнадцатый аспект изобретения относится к бактерии *Bacteroides thetaiotaomicron*, описанной в пункте 1, композиции, описанной в пункте 2 или 3, фармацевтической композиции, описанной в пункте 4 или 5, питательной добавке, описанной в пункте 6 или 7, или кормовому продукту, пищевому продукту, биологически активной пищевой добавке или пищевой добавке,

описанным в пункте 8 или 9, для производства лекарственного средства для модуляции воспаления ткани или органа у субъекта.

Шестнадцатый аспект изобретения относится к бактерии *Bacteroides thetaiotaomicron*, описанной в пункте 1, композиции, описанной в пункте 2 или 3, фармацевтической композиции, описанной в пункте 4 или 5, питательной добавке, описанной в пункте 6 или 7, или кормовому продукту, пищевому продукту, биологически активной пищевой добавке или пищевой добавке, описанным в пункте 8 или 9, для производства лекарственного средства для лечения и/или предотвращения воспалительного заболевания и/или аутоиммунного заболевания у субъекта.

Семнадцатый аспект изобретения относится к бактерии *Bacteroides thetaiotaomicron*, описанной в пункте 1, композиции, описанной в пункте 2 или 3, фармацевтической композиции, описанной в пункте 4 или 5, питательной добавке, описанной в пункте 6 или 7, или кормовому продукту, пищевому продукту, биологически активной пищевой добавке или пищевой добавке, описанным в пункте 8 или 9, для производства лекарственного средства для уменьшения разрыва толстого кишечника у субъекта, предпочтительно, в случае, когда субъект имеет IBD.

Восемнадцатый аспект изобретения относится к бактерии *Bacteroides thetaiotaomicron*, описанной в пункте 1, композиции, описанной в пункте 2 или 3, фармацевтической композиции, описанной в пункте 4 или 5, питательной добавке, описанной в пункте 6 или 7, или кормовому продукту, пищевому продукту, биологически активной пищевой добавке или пищевой добавке, описанным в пункте 8 или 9, для производства лекарственного средства для уменьшения экспрессии одного или более провоспалительных генов в клетке или клетках у субъекта.

Девятнадцатый аспект изобретения относится к бактерии *Bacteroides thetaiotaomicron*, описанной в пункте 1, композиции, описанной в пункте 2 или 3, фармацевтической композиции, описанной в пункте 4 или 5, питательной добавке, описанной в пункте 6 или 7, или кормовому продукту, пищевому продукту, биологически активной пищевой добавке или пищевой добавке, описанным в пункте 8 или 9, для производства лекарственного

средства для увеличения процентного содержания регуляторных Т-клеток (Treg) в пищеварительном тракте или секции пищеварительного тракта.

#### КРАТКОЕ ОПИСАНИЕ ФИГУР

Изобретение описано со ссылкой на сопроводительные фигуры, в которых:

**Фигура 1** показывает уменьшение степени тяжести колита за счет экспансии Treg клеток при использовании бактерии *B. thetaiotaomicron* штамма BT2013 в модели индуцированного DSS колита.

**Фигура 2** показывает, что бактерия *B. thetaiotaomicron* штамма BT2013 не влияет на Treg клетки, но влияет на Teff клетки у обычных мышей;

**Фигура 3** показывает активность миелопероксидазы (MPO) в подвздошной кишке (a) и слепой кишке (b) у мышей, получавших DSS совместно с ежедневным приемом бактерии *B. thetaiotaomicron* или без нее.

**Фигура 4** показывает результаты гистопатологического исследования восходящего отдела толстого кишечника у самок мышей C57Bl/6, получавших DSS (a) или DSS и *B. thetaiotaomicron* (b).

**Фигура 5** показывает средние баллы по гистопатологической шкале для ткани восходящего отдела толстого кишечника от мышей, кишечник которых был колонизирован бактерией *B. thetaiotaomicron* штаммов E1 и BT2013 во время индуцированного DSS колита.

**Фигура 6** показывает экспрессию провоспалительных генов (IL-1 $\beta$  и IL-6) и противовоспалительного гена (IL-10) в восходящем отделе толстого кишечника у мышей, получавших бактерию *B. thetaiotaomicron* штаммов E1, E2 и BT2013.

**Фигура 7** показывает экспрессию IL-8 в клетках Caco-2, инкубированных с РМА и средой или с бактериальными клетками E1, E2 и BT2013.

#### ПОДРОБНОЕ ОПИСАНИЕ ИЗОБРЕТЕНИЯ

Настоящее изобретение основано на том открытии, что бактерия BT штамма BT2013 оказывает более сильное противовоспалительное действие по сравнению с контрольными

штаммами ВТ.

Бактерия ВТ штамма ВТ2013 депонирована 3 декабря 2014 г. под регистрационным номером 42341 в Национальной коллекции промышленных, пищевых и морских бактерий (NCIMB) в NCIMB Ltd, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen, UK, AB21 9YA. Депонирование было произведено в соответствии с условиями Будапештской конвенции. Депонирование было произведено компанией GT Biologics Ltd. (Life Sciences Innovation Building, Aberdeen, AB25 2ZS, Scotland). GT Biologics Ltd. впоследствии была переименована в 4D Pharma Research Limited.

#### **Производное**

Настоящее изобретение охватывает производные депонированного штамма. Термин «производное» включает дочерние штаммы (потомство) или штаммы, полученные при культивировании (субклонированные) из оригинального штамма, однако модифицированные некоторым образом (в том числе, на генетическом уровне), без оказания отрицательного влияния на биологическую активность, то есть, производный штамм будет иметь по меньшей мере такую же иммуномодулирующую активность, что и оригинальный штамм ВТ2013.

#### **Биотипы**

Последовательность генома для штамма ВТ2013 приведена в SEQ ID NO: 1.

Ожидается, что бактериальные штаммы, которые являются биотипами бактерии, депонированной под регистрационным номером NCIMB 42341, также будут эффективны для лечения или предотвращения воспалительных заболеваний и/или аутоиммунных заболеваний, и/или аллергических заболеваний. Биотип представляет собой близкородственный штамм, который имеет такие же или очень схожие физиологические и биохимические характеристики.

В конкретных вариантах осуществления бактериальный штамм для применения по изобретению имеет последовательность 16s рРНК, которая по меньшей мере на 95%, 96%, 97%, 98%, 99%, 99,5% или 99,9% идентична последовательности 16s рРНК бактерии, депонированной под регистрационным номером NCIMB 42341.

Альтернативно, штаммы, которые представляют собой биотипы бактерии, депонированной под регистрационным номером NCIMB 42341, и которые подходят для применения по изобретению, могут быть идентифицированы путем секвенирования других нуклеотидных последовательностей для бактерии, депонированной под регистрационным номером NCIMB 42341. Например, практически полный геном может быть секвенирован, и штамм-биотип для применения по изобретению может иметь по меньшей мере 95%, 96%, 97%, 98%, 99%, 99,5% или 99,9% идентичности последовательностей на протяжении по меньшей мере 80% его полного генома (например, на протяжении по меньшей мере 85%, 90%, 95% или 99%, или на протяжении его полного генома). Другие подходящие последовательности для применения с целью идентификации штаммов-биотипов могут включать hsp60 или повторяющиеся последовательности, такие как BOX, ERIC, (GTG)<sub>5</sub>, или REP (Masco *et al.* (2003) *Systematic and Applied Microbiology*, 26: 557-563). Штаммы-биотипы могут иметь последовательности, имеющие по меньшей мере 95%, 96%, 97%, 98%, 99%, 99,5% или 99,9% идентичности последовательностей с соответствующей последовательностью бактерии, депонированной под регистрационным номером NCIMB 42341.

В конкретных вариантах осуществления бактериальный штамм для применения по изобретению имеет геном, имеющий идентичность последовательностей с SEQ ID NO: 1. В предпочтительных вариантах осуществления бактериальный штамм для применения по изобретению имеет геном, имеющий по меньшей мере 90% идентичности последовательностей (например, по меньшей мере 92%, 94%, 95%, 96%, 97%, 98%, 99% или 100% идентичности последовательностей) с SEQ ID NO: 1 на протяжении по меньшей мере 60% (например, по меньшей мере 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% или 100%) SEQ ID NO: 1. Например, бактериальный штамм для применения по изобретению может иметь геном, имеющий по меньшей мере 90% идентичности последовательностей с SEQ ID NO: 1 на протяжении 70% SEQ ID NO: 1, или по меньшей мере 90% идентичности последовательностей с SEQ ID NO: 1 на протяжении 80% SEQ ID NO: 1, или по меньшей мере 90% идентичности

последовательностей с SEQ ID NO: 1 на протяжении 90% SEQ ID NO: 1, или по меньшей мере 90% идентичности последовательностей с SEQ ID NO: 1 на протяжении 100% SEQ ID NO: 1, или по меньшей мере 95% идентичности последовательностей с SEQ ID NO: 1 на протяжении 70% SEQ ID NO: 1, или по меньшей мере 95% идентичности последовательностей с SEQ ID NO: 1 на протяжении 80% SEQ ID NO: 1, или по меньшей мере 95% идентичности последовательностей с SEQ ID NO: 1 на протяжении 90% SEQ ID NO: 1, или по меньшей мере 95% идентичности последовательностей с SEQ ID NO: 1 на протяжении 100% SEQ ID NO: 1, или по меньшей мере 98% идентичности последовательностей с SEQ ID NO: 1 на протяжении 70% SEQ ID NO: 1, или по меньшей мере 98% идентичности последовательностей с SEQ ID NO: 1 на протяжении 80% SEQ ID NO: 1, или по меньшей мере 98% идентичности последовательностей с SEQ ID NO: 1 на протяжении 90% SEQ ID NO: 1, или по меньшей мере 98% идентичности последовательностей с SEQ ID NO: 1 на протяжении 100% SEQ ID NO: 1.

Альтернативно, штаммы, которые являются биотипами бактерии, депонированной под регистрационным номером NCIMB 42341, и которые подходят для применения по изобретению, могут быть идентифицированы с использованием депозита под регистрационным номером NCIMB 42341 и анализа рестрикционных фрагментов и/или ПЦР-анализа, например, на основании полиморфизма длины флуоресцентно меченых амплифицированных фрагментов (FAFLP) и ПЦР-фингерпринтинга повторяющихся (rep) элементов ДНК, или белковых профилей, или частичного секвенирования 16S или 23s рДНК.

В конкретных вариантах осуществления штаммами, которые являются биотипами бактерии, депонированной под регистрационным номером NCIMB 42341, и которые подходят для применения по изобретению, являются штаммы, демонстрирующие такую же картину, что и бактерия, депонированная под регистрационным номером NCIMB 42341, при изучении методом рестрикционного анализа амплифицированной рибосомной ДНК (ARDRA), например, с использованием фермента рестрикции Sau3AI (информацию об иллюстративных методах и руководство можно найти, например, в

Srůtková *et al.* (2011) *J. Microbiol. Methods*, 87(1): 10–6). Альтернативно, штаммы-биотипы идентифицируют как штаммы, которые имеют те же характерные особенности сбраживания углеводов, что и бактерия, депонированная под регистрационным номером NCIMB 42341.

Бактериальные штаммы, которые представляют собой биотипы бактерии, депонированной под регистрационным номером NCIMB 42341, и которые могут быть использованы в композициях и способах по изобретению, могут быть идентифицированы с использованием любого подходящего метода или стратегии. Например, бактериальные штаммы, которые имеют сходные особенности роста, тип метаболизма и/или поверхностные антигены с бактерией, депонированной под регистрационным номером NCIMB 42341, могут быть полезными для изобретения. Штамм-биотип будет иметь иммуномодулирующую активность, сопоставимую с активностью штамма NCIMB 42341. Например, штамм-биотип будет проявлять эффекты в моделях индуцированного DSS колита и эффекты на уровне Treg, ферментативную активность MPO, связанную с воспалением экспрессию гена и гистопатологию толстого кишечника, сопоставимые с эффектами, наблюдаемыми в функциональных анализах, что можно определять с использованием протоколов, описанных в разделе «Функциональные анализы».

### **Заболевания**

Бактерию *Bacteroides thetaiotaomicron* штамма BT2013 можно использовать для лечения и/или предотвращения заболевания у субъекта, которое представляет собой воспалительное заболевание и/или аутоиммунное заболевание.

В одном варианте осуществления заболевание поражает пищеварительный тракт, секцию пищеварительного тракта, печень, клетки печени, иммунные клетки, эпителиальные клетки, эпидермальные клетки, нейрональные клетки, эндотелиальные клетки, фибробласты, поджелудочную железу и/или клетки поджелудочной железы (например, островки Лангерганса).

Примеры секций (то есть, частей) пищеварительного тракта включают пищевод, желудок и кишечник (такой как тонкий кишечник (например, двенадцатиперстную кишку, тощую кишку и подвздошную

кишку) и/или толстый кишечник (например, слепую кишку, восходящий отдел, поперечный отдел, нисходящий отдел и сигмовидный отдел толстого кишечника)).

Примеры эпителиальных клеток включают эпителиальные клетки кишечника. Примеры иммунных клеток включают дендритные клетки, моноциты/макрофаги, Т-клетки и нейтрофилы.

В одном варианте осуществления заболевание выбирают из группы, состоящей из:

1. заболеваний органов, таких как синдром раздраженной кишки (IBS), воспалительное заболевание кишечника, включая болезнь Крона и язвенный колит, некротизирующий энтероколит, паучит, целиакия, рассеянный склероз (головной мозг), диабет I типа, синдром Гудпасчера, тиреоидит Хашимото, хронический активный гепатит, кардиомиопатия, увеит и ринит;

2. системных заболеваний, таких как ревматоидный артрит, системная красная волчанка, склеродермия, псориаз, атопический дерматит, витилиго, рассеянный склероз, очаговая алопеция, саркоидоз, полимиозит, а также их сочетаний.

В одном аспекте заболевание поражает кишечник.

В одном аспекте заболевание представляет собой воспалительное заболевание. Например, заболевание представляет собой воспалительное заболевание кишечника (IBD), такое как болезнь Крона.

В одном аспекте заболевание представляет собой аутоиммунное заболевание. Например, аутоиммунное заболевание выбирают из группы, состоящей из язвенного колита, паучита, ревматоидного артрита, псориаза, рассеянного склероза, диабета I типа, аллергии (включая целиакию), атопического дерматита и ринита.

### **Субъект**

В одном варианте осуществления субъект является моногастрическим животным.

Примеры моногастрических животных включают домашнюю птицу, людей, крыс, свиней, собак, кошек, лошадей и кроликов.

В другом варианте осуществления субъект является млекопитающим, таким как моногастрическое млекопитающее.

Примеры моногастрических млекопитающих включают всеядных



животных (таких как люди, крысы и свиньи), плотоядных животных (таких как собаки и кошки) и травоядных животных (таких как лошади и кролики).

Предпочтительно, субъект является человеком.

В одном аспекте субъект имеет заболевание, выбранное из группы, состоящей из воспалительного заболевания кишечника (IBD), колита, ревматоидного артрита, псориаза, рассеянного склероза, диабета I типа, целиакии, атопического дерматита, ринита, синдрома раздраженной кишки (IBS), язвенного колита, паучита, болезни Крона, функциональной диспепсии, атопических заболеваний, некротизирующего энтероколита, болезни неалкогольной жировой инфильтрации печени, желудочно-кишечной инфекции, а также их сочетаний. Например, субъект имеет IBD.

#### **Модуляция/регуляция**

В настоящем документе термины «модуляция» и «регуляция» могут быть использованы взаимозаменяемо.

В одном варианте осуществления бактерию *B. thetaiotaomicron* штамма BT2013 используют для модуляции воспаления клетки, ткани или органа у субъекта.

В одном варианте осуществления термин «модуляция» означает увеличение и/или индукцию, и/или стимуляцию, и/или активацию. В альтернативном варианте осуществления термин «модуляция» означает уменьшение и/или сокращение, и/или ингибирование.

В одном варианте осуществления термин «регуляция» означает повышающую регуляцию. В альтернативном варианте осуществления термин «регуляция» означает понижающую регуляцию.

В одном варианте осуществления бактерия *B. thetaiotaomicron* штамма BT2013, описанная в настоящем документе, способствуют уменьшению воспаления клетки, ткани или органа. Например, воспаление пищеварительного тракта, секции (то есть, части) пищеварительного тракта (например, кишечника), печени, клеток печени, эпителиальных клеток, эпидермальных клеток, нейрональных клеток, эндотелиальных клеток, фибробластов, поджелудочной железы и/или клеток поджелудочной железы (например, островков Лангерганса) уменьшается.

В одном примере воспаление пищеварительного тракта или его

части (например, кишечника) уменьшается.

В другом примере воспаление иммунных клеток ткани или органа уменьшается.

В другом примере воспаление эпителиальных клеток ткани или органа уменьшается.

Используемый в настоящем документе термин «воспаление» означает одно или более из следующего: покраснение, набухание, боль, чувствительность, жар и нарушение функции клетки, ткани или органа из-за воспалительного процесса, запускаемого в результате чрезмерной реакции иммунной системы.

В одном варианте осуществления число клеток, которые воспалены у субъекта, по меньшей мере на 10%, 20%, 30%, 40% или 50% ниже после введения штамма BT2013, описанного в настоящем документе, по сравнению с числом клеток, которые воспалены у субъекта до того, как штамм BT2013, описанный в настоящем документе, вводят субъекту.

В одном варианте осуществления количество ткани или органа, которые воспалены у субъекта, по меньшей мере на 10%, 20%, 30%, 40% или 50% ниже после введения штамма BT2013 по сравнению с количеством ткани или органа, которые воспалены у субъекта, до того, как штамм BT2013 вводят субъекту.

В одном варианте осуществления штамм BT2013 способствует уменьшению воспаления эпителиальных клеток ткани или органа.

Например, эпителиальные клетки представляют собой эпителиальные клетки пищеварительного тракта или его части (например, кишечника).

Без привязки к конкретной теории, штамм BT2013 способствует увеличению продуцирования Т-клеток (таких как регуляторные Т-клетки, которые также могут быть названы Treg) у субъекта. Это увеличение количества Treg может приводить к ослаблению эффектов других эффекторных Т-клеток (также называемых Teff), таких как Th1, Th17 и Th2, которые вызывают воспаление, аутоиммунные и аллергические/атопические состояния. При болезни Крона и язвенном колите нарушен баланс Teff/Treg клеток.

В одном варианте осуществления продуцирование Т-клеток у субъекта увеличивается так, что имеется по меньшей мере на 10%,

20%, 30%, 40% или 50% больше Т-клеток, или более чем на 100% больше Т-клеток после введения штамма BT2013, описанного в настоящем документе, по сравнению с количеством Т-клеток у субъекта до того, как штамм BT2013 вводят субъекту.

#### **Целостность кишечного барьера**

В одном варианте осуществления штамм BT2013 используют для повышения целостности кишечного барьера у субъекта.

Используемый в настоящем документе термин «повышение целостности кишечного барьера» означает уменьшение количеств и/или видов микроорганизмов, которые распространяются из кишечника в другие клетки субъекта, после введения штамма BT2013 по сравнению с количествами и/или видами микроорганизмов, которые распространяются из кишечника в другие клетки субъекта, до введения штамма BT2013, описанного в настоящем документе.

В одном варианте осуществления количества микроорганизмов, которые распространяются из кишечника в другие клетки субъекта, становятся по меньшей мере на 10%, 20%, 30%, 40% или 50% ниже после введения штамма BT2013 по сравнению с количествами микроорганизмов, которые распространяются из кишечника в другие клетки субъекта до введения.

В одном варианте осуществления имеется по меньшей мере на 5%, 10%, 15% или 20% меньше видов микроорганизмов, которые распространяются из кишечника в другие клетки субъекта, после введения штамма BT2013 по сравнению с видами микроорганизмов, которые распространяются из кишечника в другие клетки субъекта, до введения.

#### **Разрыв кишечника**

В одном варианте осуществления штамм BT2013 используют для уменьшения разрыва кишечника (например, толстого кишечника) у субъекта (например, субъекта с IBD).

Используемый в настоящем документе термин «разрыв кишечника у субъекта» означает нарушение целостности эпителия слизистой оболочки и/или изменение количества бокаловидных клеток в эпителии, и/или изменение количества иммунных клеток, инфильтрирующих собственную пластинку слизистой оболочки кишечника.

В одном варианте осуществления штамм BT2013 способствует уменьшению или предотвращению нарушения целостности эпителия слизистой оболочки и/или способствует уменьшению или предотвращению сокращения количества бокаловидных клеток в эпителии, и/или способствует уменьшению или предотвращению инфильтрации иммунных клеток в собственную пластинку слизистой оболочки кишечника.

В одном варианте осуществления уменьшение нарушения целостности эпителия слизистой оболочки представляет собой уменьшение по меньшей мере на 5%, 10%, 15% или 20% количества бактерий, выходящих из просвета кишечника в клетки кишечника, после введения штамма BT2013 по сравнению с количеством бактерий, выходящих из просвета кишечника в клетки кишечника, у субъекта до введения.

В одном варианте осуществления уменьшение количества бокаловидных клеток в эпителии представляет собой уменьшение по меньшей мере на 2%, 5%, 10%, 15% или 20% количества бокаловидных клеток в эпителии у субъекта после введения штамма BT2013 по сравнению с количеством бокаловидных клеток в эпителии у субъекта до введения.

В одном варианте осуществления уменьшение инфильтрации иммунных клеток в собственную пластинку слизистой оболочки кишечника является таким, что за определенный период времени (например, 24 часа) имеет место уменьшение по меньшей мере на 5%, 10%, 15%, 20% или 30% количества иммунных клеток (например, Т-клеток), проникающих в собственную пластинку слизистой оболочки кишечника, после введения штамма BT2013 по сравнению с количеством иммунных клеток (например, Т-клеток), проникающих в собственную пластинку слизистой оболочки кишечника, у субъекта до введения.

#### **Провоспалительные гены и гены, влияющие на целостность барьера**

В одном варианте осуществления штамм BT2013 используют для регуляции экспрессии одного или более провоспалительных генов и/или одного или более генов, влияющих на целостность барьера, в клетке или клетках у субъекта.

В одном варианте осуществления термин «регуляция» означает повышающую регуляцию экспрессии одного или более провоспалительных генов. В альтернативном варианте осуществления термин «регуляция» означает понижающую регуляцию экспрессии одного или более провоспалительных генов.

В одном варианте осуществления штамм BT2013 способствует понижающей регуляции экспрессии одного или более провоспалительных генов в клетке или клетках у субъекта.

Используемый в настоящем документе термин «провоспалительный ген» означает ген, который, при его экспрессии, стимулирует воспаление. Примеры провоспалительных генов включают гены, кодирующие, но без ограничения, IL1- $\beta$ , IL4, IL5, IL6, IL8, IL12, IL13, IL17, IL21, IL22, IL23, IL27, IFN, CCL2, CCL3, CCL5, CCL20, CXCL5, CXCL10, CXCL12, CXCL13 и TNF- $\alpha$ .

В одном варианте осуществления провоспалительный ген выбирают из группы, состоящей из IL1- $\beta$ , IL6 и IL8.

В одном варианте осуществления уровень экспрессии (например, уровень мРНК) одного или более провоспалительных генов снижается (то есть, имеет место понижающая регуляция) так, что уровень становится по меньшей мере на 10%, 20%, 30%, 40% или 50% ниже после введения штамма BT2013 по сравнению с уровнем у субъекта до введения.

Используемый в настоящем документе термин «гены, влияющие на целостность барьера» означает ген, который, при его экспрессии, играет роль в функции барьера кишечника, такой как восстановление барьера и предотвращение проникновения микроорганизмов за барьер. Примеры генов, влияющих на целостность барьера, включают гены, кодирующие Retnlg|Retnlb, Si, Defa24, Hsd11b2, Hsd17b2 и Nr1d1|Thra.

В одном варианте осуществления термин «регуляция» означает повышающую регуляцию экспрессии одного или более генов, влияющих на целостность барьера. В альтернативном варианте осуществления термин «регуляция» означает понижающую регуляцию экспрессии одного или более генов, влияющих на целостность барьера.

В одном варианте осуществления штамм BT2013 способствует

повышающей регуляции экспрессии генов, влияющих на целостность барьера, в клетке или клетках у субъекта.

В одном варианте осуществления ген, влияющий на целостность барьера, выбирают из группы, состоящей из Retnlg|Retnlb, Si, Defa24, Hsd11b2, Hsd17b2 и Nr1d1|Thra.

В одном варианте осуществления уровень экспрессии (например, уровень мРНК) одного или более генов, влияющих на целостность барьера, повышается (то есть, имеет место повышающая регуляция) так, что уровень становится по меньшей мере на 10%, 20%, 30%, 40% или 50% выше после введения штамма VT2013 по сравнению с уровнем у субъекта до введения.

#### **Пищеварительный тракт**

Части пищеварительного тракта включают пищевод, желудок и кишечник (такой как тонкий кишечник (например, двенадцатиперстную кишку, тощую кишку и подвздошную кишку) и/или толстый кишечник (например, слепую кишку, восходящий отдел, поперечный отдел, нисходящий отдел и сигмовидный отдел толстого кишечника)).

В настоящем документе термин «толстый кишечник» может быть использован взаимозаменяемо с термином «толстая кишка».

В одном варианте осуществления штамм VT2013 используют для улучшения состояния пищеварительного тракта у субъекта.

Используемый в настоящем документе термин «улучшение состояния пищеварительного тракта» означает уменьшение уровня воспаления в пищеварительном тракте или его части и/или улучшение микробиоты кишечника.

В одном варианте осуществления уровень воспаления в пищеварительном тракте становится по меньшей мере на 10%, 20%, 30%, 40% или 50% ниже после введения штамма VT2013 по сравнению с уровнем воспаления в пищеварительном тракте у субъекта до введения.

В одном варианте осуществления штамм VT2013 используют для улучшения микробиоты кишечника у субъекта.

Используемый в настоящем документе термин «микробиота кишечника» означает микроорганизмы, которые живут в пищеварительном тракте животных-хозяев. Эти микроорганизмы

выполняют самые различные метаболические, структурные, защитные и другие полезные функции.

Используемый в настоящем документе термин «улучшение микробиоты кишечника» означает увеличение количества и/или видов желательных микроорганизмов, присутствующих в кишечнике у субъекта (например, хозяина), и/или увеличение активности указанных желательных микроорганизмов при выполнении их метаболических, структурных, защитных и других полезных функций. Термин «улучшение микробиоты кишечника» также может означать уменьшение количества и/или видов нежелательных микроорганизмов, присутствующих в кишечнике у субъекта (например, хозяина), и/или уменьшение активности указанных нежелательных микроорганизмов при выполнении их метаболических, структурных, защитных и других полезных функций.

Микроорганизмы, присутствие которых желательно в кишечнике хозяина, являются такими микроорганизмами, которые выполняют защитные и полезные функции. Бактерии *Firmicutes* и/или *Bacteroidetes* являются примерами желательных микроорганизмов в кишечнике хозяина.

Микроорганизмы, присутствие которых нежелательно в кишечнике хозяина, являются такими микроорганизмами, которые могут препятствовать выполнению метаболических, структурных, защитных и других полезных функций желательными микроорганизмами в кишечнике. Дополнительно или альтернативно, нежелательными микроорганизмами являются такие, которые вызывают, например, воспаление и/или диарею. *E. coli* является примером нежелательного микроорганизма в кишечнике хозяина.

Например, изменение баланса между желательными микроорганизмами (такими как бактерии *Firmicutes* и/или *Bacteroidetes*) и нежелательными микроорганизмами (такими как *E.coli*: ETEC, EPEC, EIEC, EHEC и EAEC) в микробиоте кишечника может происходить у субъектов с воспалительным заболеванием кишечника (IBD), после того как штамм BT2013 был введен субъекту.

В одном варианте осуществления количество желательных микроорганизмов (таких как бактерии *Firmicutes* и/или

*Bacteroidetes*), присутствующих в кишечнике у субъекта (например, хозяина), увеличивается так, что количество микроорганизмов становится по меньшей мере на 10%, 20%, 30%, 40% или 50% больше, или более чем на 100% больше после введения штамма BT2013 по сравнению с уровнем у субъекта до введения. Дополнительно или альтернативно, число видов желательных микроорганизмов (таких как бактерии *Firmicutes* и/или *Bacteroidetes*), присутствующих в кишечнике у субъекта (например, хозяина), увеличивается так, что становится по меньшей мере на 2%, 5%, 10%, или 15% больше видов микроорганизмов после введения штамма BT2013 по сравнению с видами микроорганизмов у субъекта до введения.

В одном варианте осуществления количество нежелательных микроорганизмов (таких как *E. coli* ETEC, EPEC, EIEC, EHEC и EAEC), присутствующих в кишечнике у субъекта (например, хозяина), уменьшается так, что количество микроорганизмов становится по меньшей мере на 10%, 20%, 30%, 40% или 50% ниже после введения штамма BT2013 по сравнению с уровнем у субъекта до введения. Дополнительно или альтернативно, число видов нежелательных микроорганизмов (таких как *E. coli* ETEC, EPEC, EIEC, EHEC и EAEC), присутствующих в кишечнике у субъекта (например, хозяина), уменьшается так, что становится по меньшей мере на 1%, 2%, 5%, или 10% меньше видов нежелательных микроорганизмов после введения штамма BT2013 по сравнению с видами микроорганизмов у субъекта до введения.

### **Инкапсуляция**

В одном варианте осуществления бактерия *B. thetaiotaomicron* штамма BT2013 является инкапсулированной.

В следующем варианте осуществления фармацевтическая композиция, содержащая штамм BT2013, является инкапсулированной.

В другом варианте осуществления питательная добавка, содержащая штамм BT2013, является инкапсулированной.

В следующем варианте осуществления кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка, описанные в настоящем документе, являются инкапсулированными.

Используемый в настоящем документе термин «инкапсуляция»



означает средство защиты штамма BT2013 от несовместимой среды путем физического разделения таким образом, что он может быть доставлен в целевой участок (например, кишечник) без деградации или существенной деградации с тем, чтобы штамм BT2013 мог проявлять свой эффект в целевом участке. Примером является капсула с энтеросолюбильным покрытием или энтеросолюбильная капсула.

Даже если целью инкапсуляции является изоляция штамма от окружающей его среды, защитное покрытие или оболочка должны разрываться во время оказания желательного действия. Разрыв защитного покрытия или оболочки, как правило, происходит в результате действия химических и физических факторов, таких как давление, атака ферментов, химическая реакция и физический распад.

Например, инкапсуляция является гарантией того, что штамм может быть принят внутрь с тем, чтобы микроорганизмы могли быть доставлены в целевой участок (например, кишечник) в количестве, которое эффективно для оказания воздействия в целевом участке.

#### **Фармацевтическая композиция**

В одном варианте осуществления фармацевтическая композиция содержит микроорганизмы штамма BT2013 и, необязательно, фармацевтически приемлемый эксципиент, носитель или разбавитель.

Фармацевтическая композиция может быть любой фармацевтической композицией. В одном аспекте фармацевтическая композиция предназначена для перорального, энтерального или ректального введения. Например, композиция может быть съедобной композицией. «Съедобным» считают материал, который одобрен для приема внутрь человеком или животным.

Фармацевтические композиции могут быть предназначены для применения людьми и животными в медицинской и ветеринарной практике.

Примеры подходящих эксципиентов для различных форм фармацевтических композиций, описанных в настоящем документе, можно найти в «Handbook of Pharmaceutical Excipients, 2-е издание, (1994), под редакцией A. Wade и P.J. Weller.

Приемлемые носители или разбавители для терапевтического

применения хорошо известны в фармацевтической области и описаны, например, в Remington's Pharmaceutical Sciences, Mack Publishing Co. (под редакцией A. R. Gennaro, 1985).

Примеры подходящих носителей включают лактозу, крахмал, глюкозу, метилцеллюлозу, стеарат магния, маннит, сорбит и тому подобное.

Примеры подходящих разбавителей включают одно или более из: воды, этанола, глицерина, пропиленгликоля и глицерина, а также их сочетания.

Фармацевтический носитель, эксципиент или разбавитель можно выбрать на основании намеченного пути введения и стандартной фармацевтической практики. Фармацевтические композиции могут содержать в качестве, или помимо, носителя, эксципиента или разбавителя любое подходящее связывающее вещество (а), смазывающее средство (а), суспендирующее средство (а), покрывающее средство (а), солюбилизующее средство (а).

Примеры подходящих связывающих веществ включают крахмал, желатин, природные сахара, такие как глюкоза, безводная лактоза, свободно сыпучая лактоза, бета-лактоза, кукурузные подсластители, природные и синтетические камеди, такие как гуммиарабик, трагакант или альгинат натрия, карбоксиметилцеллюлозу и полиэтиленгликоль.

Примеры подходящих смазывающих средств включают олеат натрия, стеарат натрия, стеарат магния, бензоат натрия, ацетат натрия, хлорид натрия и тому подобное.

В фармацевтическую композицию можно включать консерванты, стабилизаторы, красители и даже вкусовые добавки. Примеры консервантов включают бензоат натрия, сорбиновую кислоту и сложные эфиры п-гидроксibenзойной кислоты. Также можно использовать антиоксиданты и суспендирующие средства.

В одном аспекте микроорганизмы штамма BT2013 в фармацевтической композиции являются инкапсулированными.

Фармацевтический препарат может быть в форме раствора или в твердой форме - в зависимости от применения и/или способа нанесения, и/или способа введения.

Используемый в настоящем документе термин «лекарственное

средство» охватывает лекарственные средства, используемые как для людей, так и для животных, в медицинской и ветеринарной практике. Кроме того, используемый в настоящем документе термин «лекарственное средство» означает любое вещество, которое обеспечивает терапевтический и/или полезный эффект. Используемый в настоящем документе термин «лекарственное средство» не обязательно ограничен веществами, для которых необходимо разрешение на продажу, но также может включать вещества, которые могут быть использованы в косметике, нутрицевтиках, пищевых продуктах (включая, например, корма и напитки), пробиотических культурах, питательных добавках и натуральных лечебных средствах. Кроме того, используемый в настоящем документе термин «лекарственное средство» охватывает продукты, предназначенные для включения в корма для животных, например, корма для сельскохозяйственных животных и/или корма для домашних животных.

#### **Питательные добавки**

Приемлемые для питания носители, разбавители и эксципиенты включают те, которые подходят для употребления людьми и животными, и которые стандартно используют в пищевой промышленности. Типичные приемлемые для питания носители, разбавители и эксципиенты известны специалистам в данной области.

В одном варианте осуществления питательная добавка содержит микроорганизмы штамма BT2013 или клетку-хозяина, содержащую экспрессионный вектор, содержащий указанную полинуклеотидную последовательность, и приемлемый для питания эксципиент, носитель или разбавитель.

В одном примере микроорганизмы штамма BT2013 являются инкапсулированными.

#### **Кормовые продукты/продукты**

Следующий аспект изобретения относится к кормовым продуктам, пищевым продуктам, биологически активным пищевым добавкам и пищевым добавкам, содержащим микроорганизмы штамма BT2013.

Используемые в настоящем документе термины «кормовой продукт», «пищевой продукт», «пищевая добавка» и «биологически

активная пищевая добавка» охватывают все предназначенные для потребления продукты, которые могут быть твердыми, желеобразными или жидкими.

Термин «пищевой продукт» используется в широком смысле – и включает пищевые продукты для людей, а также пищевые продукты для животных (то есть, корм). В одном аспекте пищевой продукт предназначен для потребления человеком. Примеры пищевых продуктов включают молочные продукты (такие как молоко, сыр, напитки, содержащие белок молочной сыворотки, молочные напитки, молочнокислые напитки, йогурт, питьевой йогурт), хлебобулочные изделия, напитки и порошковые напитки.

«Кормовой продукт», «пищевой продукт», «пищевая добавка» и «биологически активная пищевая добавка» могут находиться в форме раствора или быть твердыми – в зависимости от применения и/или способа нанесения, и/или способа введения.

Используемый в настоящем документе термин «биологически активная пищевая добавка» включает препарат, который добавлен или может быть добавлен в пищевой продукт или кормовой продукт в качестве питательной добавки. Используемый в настоящем документе термин «биологически активная пищевая добавка» также относится к препаратам, которые могут быть использованы в небольших количествах в самых разнообразных продуктах, которые требуют желирования, текстурирования, стабилизации, суспендирования, пленкообразования и структурирования, сохранения сочности и улучшения вкуса, без добавления вязкости.

Соответствующие пищевые продукты могут включать, например, функциональные пищевые продукты, пищевые композиции, корм для домашних животных, корма для сельскохозяйственных животных, продукты здорового питания, кормовые продукты и тому подобное. В одном аспекте пищевой продукт представляет собой продукт здорового питания.

Используемый в настоящем документе термин «функциональный пищевой продукт» означает пищевой продукт, который обладает не только питательной ценностью, но также способен обеспечивать дополнительный положительный эффект для потребителя. Соответственно, функциональные пищевые продукты представляют

собой обычные пищевые продукты, которые имеют компоненты или ингредиенты (такие как те, которые описаны в настоящем документе) в их составе, придающие пищевому продукту конкретное функциональное – например, лечебное или физиологическое – преимущество, кроме чисто питательной ценности.

Примеры конкретных пищевых продуктов, которые можно использовать по настоящему изобретению, включают молочные продукты, готовые к употреблению десерты, порошки для разведения, например, в молоке или воде, шоколадные молочные напитки, солодовые напитки, готовые блюда, блюда и напитки быстрого приготовления для людей или пищевые композиции, полностью или частично составляющие диету для домашних или сельскохозяйственных животных.

В одном аспекте кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка по настоящему изобретению предназначены для людей, домашних животных или сельскохозяйственных животных, таких как моногастрические животные. Кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка могут быть предназначены для животных, выбранных из группы, состоящей из собак, кошек, свиней, лошадей или домашней птицы. В следующем варианте осуществления пищевой продукт, биологически активная пищевая добавка или пищевая добавка предназначена для взрослых особей, в частности, взрослых людей.

Используемый в настоящем документе термин «молочный продукт» означает любой жидкий или полутвердый продукт на основе молока или молочной сыворотки, имеющий различное содержание жира. Молочными продуктами могут быть, например, коровье молоко, козье молоко, овечье молоко, снятое молоко, цельное молоко, молоко, восстановленное из порошкового молока, и молочная сыворотка без какой бы то ни было обработки, либо обработанный продукт, такой как йогурт, простокваша, творог, кислое молоко, цельное кислое молоко, пахта и другие кисломолочные продукты. Другая важная группа включает молочные напитки, такие как напитки из молочной сыворотки, ферментированное молоко, стуженное молоко, молочные напитки для младенцев и детей; молоко

с ароматическими добавками, мороженое; содержащие молоко продукты, такие как сладости.

Кормовые продукты, пищевые продукты, биологически активные пищевые добавки или пищевые добавки по настоящему изобретению могут представлять собой - или могут быть добавлены в - биологически активные добавки, также называемые в настоящем документе диетическими или питательными добавками, или пищевыми добавками.

Кормовые продукты, пищевые продукты, биологически активные пищевые добавки или пищевые добавки по настоящему изобретению также могут быть использованы в кормах для животных (например, в кормах для свиней), особенно в ранний период отлучения от матери и период роста и откармливания. Ожидается, что кормовые продукты, пищевые продукты, биологически активные пищевые добавки или пищевые добавки будут способствовать усилению иммунитета, уменьшению и предотвращению инфекционных заболеваний, созданию более полезного состава микробиоты, а также увеличению роста и производительности животных, например, за счет повышения эффективности усвоения пищи.

В одном варианте осуществления кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка являются инкапсулированными.

#### **Живой биотерапевтический препарат**

Микроорганизмы штамма BT2013 могут быть использованы в составе, или в качестве, живого биотерапевтического препарата (LBP).

В одном аспекте LBP представляет собой вводимую перорально композицию метаболически активных, то есть, живых и/или лиофилизированных, либо нежизнеспособных убитых нагреванием, облучением или лизированных бактерий. LBP может содержать и другие ингредиенты. LBP можно вводить перорально, то есть, в форме таблетки, капсулы или порошка. Кроме того, LBP может содержать и другие виды бактерий, например, вид бактерий *R. hominis*. В случае бактерии *R. hominis* предпочтительны инкапсулированные препараты, поскольку она является анаэробной. Другие ингредиенты (такие как витамин С, например) можно

включать в качестве поглотителей кислорода и субстратов (например, тех, которые способствуют колонизации и выживанию *in vivo*). Альтернативно, LBP по изобретению можно вводить перорально в качестве пищевого или питательного продукта, такого как кисломолочный продукт на основе молока или молочной сыворотки, либо в качестве фармацевтического препарата.

Подходящая суточная доза бактерий в LBP составляет от примерно  $1 \times 10^3$  до примерно  $1 \times 10^{12}$  колониеобразующих единиц (КОЕ); например, от примерно  $1 \times 10^7$  до примерно  $1 \times 10^{10}$  КОЕ; в другом примере от примерно  $1 \times 10^6$  до примерно  $1 \times 10^{10}$  КОЕ.

В одном аспекте LBP содержит бактерии и/или их клеточные компоненты в качестве активных ингредиентов в количестве от примерно  $1 \times 10^6$  до примерно  $1 \times 10^{12}$  КОЕ/г, в расчете на массу композиции; например, от примерно  $1 \times 10^8$  до примерно  $1 \times 10^{10}$  КОЕ/г. Как правило, LBP, необязательно, комбинируют с по меньшей мере одним подходящим пребиотическим соединением. Как правило, пребиотик представляет собой неусваиваемый углевод, такой как олиго- или полисахарид, или сахарный спирт, который не распадается или не поглощается в верхних отделах пищеварительного тракта. Известные пребиотики включают коммерческие продукты, такие как инулин и трансгалактоолигосахариды.

В одном аспекте LBP по настоящему изобретению содержит пребиотик в количестве от примерно 1 до примерно 30% по массе в расчете на общую массу композиции (например, от 5 до 20% по массе). Углеводы можно выбирать из группы, состоящей из: фруктоолигосахаридов (или FOS), фруктоолигосахаридов с короткой цепью, инулина, изомальтолигосахаридов, пектинов, ксилоолигосахаридов (или XOS), хитозанолигосахаридов (или COS), бета-глюканов, арабийской камеди, модифицированных и устойчивых крахмалов, полидекстрозы, D-тагатозы, волокон арабийской камеди, волокон рожкового дерева, овса и цитрусовых. В одном аспекте пребиотики представляют собой фруктоолигосахариды с короткой цепью (для простоты далее в настоящем документе их называют FOSs-c.c); указанные FOSs-c.c. представляет собой неусваиваемые

углеводы, как правило, получаемые путем переработки свекловичного сахара и содержащие молекулу сахарозы, с которой связаны три молекулы глюкозы.

### **Введение**

Фармацевтические композиции, питательные добавки, кормовые продукты, пищевые продукты, биологически активные пищевые добавки или пищевые добавки по настоящему изобретению могут быть приспособлены для перорального, ректального, вагинального, парентерального, внутримышечного, внутрибрюшинного, внутриартериального, интратекального, внутрибронхиального, подкожного, внутрикожного, внутривенного, назального, трансбуккального или подъязычного путей введения.

В одном аспекте фармацевтические композиции, питательные добавки, кормовые продукты, пищевые продукты, биологически активные пищевые добавки или пищевые добавки по настоящему изобретению приспособлены для перорального, ректального, вагинального, парентерального, назального, трансбуккального или подъязычного путей введения.

В следующем аспекте фармацевтические композиции, питательные добавки, кормовые продукты, пищевые продукты, биологически активные пищевые добавки или пищевые добавки по настоящему изобретению приспособлены для перорального введения.

Для перорального введения особенно часто используют прессованные таблетки, пилюли, таблетки, гелевые капсулы, капли и капсулы.

Другие формы для введения включают растворы или эмульсии, которые можно вводить внутривенно, внутриартериально, интратекально, подкожно, внутрикожно, внутрибрюшинно или внутримышечно, и которые готовят из стерильных или стерилизуемых растворов. Фармацевтические композиции по настоящему изобретению также могут иметь форму суппозиторий, pessaries и суспензий. Фармацевтические композиции, питательные добавки, кормовые продукты, пищевые продукты, биологически активные пищевые добавки или пищевые добавки могут быть сформулированы в стандартной лекарственной форме, то есть, в форме отдельных порций, содержащих стандартную дозу, либо несколько стандартных



доз или часть стандартной дозы.

### **Дозировка**

Рядовой специалист в данной области может с легкостью и без излишнего экспериментирования определять соответствующую дозу штамма BT2013 для введения субъекту. Как правило, врач определяет фактическую дозу, которая наилучшим образом подойдет для конкретного пациента, и она будет зависеть от различных факторов, включая активность используемого бактериального штамма, метаболическую стабильность и продолжительность действия данного штамма, возраст, массу тела, общее состояние здоровья, пол, диету, способ и время введения, скорость выведения, сочетание лекарственных средств, степень тяжести конкретного состояния у индивидуума, получающего лечение. Дозы, раскрытые в настоящем документе, являются иллюстративными для усредненного случая. Безусловно, могут иметь место конкретные обстоятельства, когда следует использовать более высокие или низкие дозы, и такие дозы входят в объем данного изобретения.

### **Сочетания**

В одном аспекте микроорганизмы штамма BT2013 вводят в сочетании с одним или более другими активными средствами. В таких случаях микроорганизмы штамма BT2013 можно вводить подряд, одновременно или последовательно с одним или более другими активными средствами.

### **Функциональные анализы:**

#### *In vivo* модель

Мышей C57BL/6 (в возрасте 6 недель) использовали для оценки терапевтического эффекта бактерий *B. thetaiotaomicron* штаммов E1, E2 и BT2013 во время индуцированного DSS колита. Перед применением DSS проводили колонизацию кишечника мышей бактериями одного из штаммов *B. thetaiotaomicron*. Животных подвергали эвтаназии и собирали образцы ткани кишечника. Тонкий кишечник собирали для иммунологического анализа методом проточной цитометрии и измерения ферментативной активности фермента миелопероксидазы (MPO). Восходящий отдел толстого кишечника делили на равные части и переносили в нейтральный забуференный формалин (NBF; Sigma-Aldrich) для гистологического анализа или в

RNAlater (Ambion) для молекулярного анализа.

Проводили анализ методом проточной цитометрии популяций Т-клеток в собственной пластинке слизистой оболочки тонкого кишечника (фигура 1 и фигура 2). Воздействие только DSS и *B. thetaiotaomicron* не влияло на общее процентное содержание популяции CD3+CD4+CD8- клеток. Популяциями, на которые оказывали воздействие только DSS и *B. thetaiotaomicron*, были клетки Treg (CD25+FoxP3+\* и FR4<sup>hi</sup>CD25+\*) и клетки Teff (FR4<sup>lo</sup>CD25+\*) (фигура 1 и 2). Процентное содержание Treg было повышено у мышей, получавших бактерии *B. thetaiotaomicron* штамма BT2013, по сравнению с мышами, получавшими только DSS. Судя по всему, штамм E1W не оказывал какого-либо влияния на Treg (фигура 1). Эффекты BT2013 на Treg были заметны только у мышей, получавших эти бактерии совместно с DSS. Штамм не проявлял эффект в отношении Treg у не подвергнутых воздействию мышей, но оказывал влияние на популяцию клеток Teff (фигура 2).

Определяли ферментативную активность MPO в подвздошной кишке и слепой кишке (фигура 3a и 3b). MPO представляет собой провоспалительный фермент, хранящийся в азурофильных гранулах нейтрофильных гранулоцитов. MPO используют в качестве индикатора воспаления, особенно рекрутинга и накопления нейтрофилов. Пониженные уровни активности MPO, измеренной в образцах ткани подвздошной или слепой кишки от мышей, получавших *B. thetaiotaomicron*/DSS, по сравнению с мышами, получавшими только DSS, свидетельствовали об уменьшении рекрутинга нейтрофилов и, следовательно, уменьшении воспаления.

Проводили гистологический анализ восходящего отдела толстого кишечника (фигуры 4 и 5 и таблица 1). Система присвоения баллов по гистопатологической шкале была основана на критерии, описанном в Berg *et al.*, 1996, вкратце изложенном далее:

0=Неглубокие крипты, инфильтрация воспалительных клеток отсутствует или незначительная, интактный эпителий, бокаловидные клетки выглядят заполненными муцином, то есть, без патологии

1=В криптах может наблюдаться небольшая гиперплазия эпителиальных клеток, можно видеть несколько диффузно

инфильтрировавших воспалительных клеток между криптами, эпителий в просвете кишечника выглядит незатронутым, бокаловидные клетки могут выглядеть содержащими немного меньше муцина.

2=Крипты выглядят глубже с четкими признаками эпителиальной гиперплазии, в бокаловидных клетках недостаточно муцина, четко видна инфильтрация воспалительных клеток, которая может быть многоочаговой по характеру, хотя инфильтраты не видны в подслизистой оболочке.

3=Лезии захватывают большую площадь слизистой оболочки и/или встречаются чаще, чем при степени 2. Лезии не захватывают подслизистую оболочку. Эпителиальные клетки в просвете кишечника демонстрируют небольшую эрозию. Лезии не являются трансмуральными.

4=Эпителий крипт выглядит эродированным. Могут присутствовать абсцессы. Эпителиальные клетки в просвете кишечника выглядят необычно, иногда полностью утрачены. Наблюдается трансмуральный инфильтрат – это часто связано с полной потерей эпителиальных клеток в просвет кишечника.

Разрыв толстого кишечника вследствие индуцированного DSS колита был значительно меньше в случае лечения мышей бактериями *B. thetaiotaomicron* штаммов E1, E2 и BT2013. Экспрессия связанных с воспалением генов в восходящем отделе толстого кишечника была снижена у мышей, кишечник которых был колонизирован бактериями *B. thetaiotaomicron*, по сравнению с мышами, получавшими только DSS. Бактерии штаммов E1 и BT2013 гораздо сильнее уменьшали экспрессию воспалительных генов IL1 $\beta$  и IL6, по сравнению с бактериями штамма E2. (фигура 6)

**Таблица 1**

<u>TTEST</u>	<u>КОНТРОЛЬ</u>	<u>BT E1</u>	<u>BT 2013</u>	<u>BT E2</u>
<u>DSS</u>	<u>0,000</u>	<u>0,032</u>	<u>0,041</u>	<u>0,089</u>

In vitro модель

Экспрессия воспалительного гена интерлейкина-8, индуцированная в эпителиальных клетках кишечника после воздействия PMA, была модулированной в присутствии бактерий *B. thetaiotaomicron* штаммов E1, E2 и BT2013 (фигура 7).

## Секвенирование генома штамма BT2013

Проводили секвенирование образца ДНК из штамма BT2013 на MiSeq (v2 нано 2×250 п.н.) с использованием библиотеки Nextera XT для быстрой фрагментации и тегирования адаптерами для секвенирования, получая в общей сложности 4605120 прочтений (1115615927 нуклеотидов).

Сводная информация об анализе данных приведена ниже:

- a. Картирование на контрольную последовательность (NC\_004663 и NC\_004703) с использованием bowtie2 (2,2,2)
- b. Идентификация SNV и малых InDel с использованием VarScan (2.3.7) и SNVer (0.5.3), выполняющих распознавание консенсуса во избежание ложноположительных результатов
- c. Аннотация вариаций с использованием контрольного gff
- d. Идентификация больших InDel с использованием pindel (0.2.5a3)
- e. *De-novo* сборка некартированных прочтений с использованием SOAPdenovo (2.04)
- f. Blast собранных контигов против базы данных нуклеотидных последовательностей NCBI
- g. Подвыборка всех прочтений выборки до 50%
- h. *De-novo* сборка на прочтениях подвыборки с использованием SOAPdenovo (2.04).

Последовательности картировали на контрольную последовательность (NC\_004663 и NC\_004703) с использованием bowtie2 (2.2.2). Нуклеотидные вариации и небольшие вставки и/или делеции идентифицировали с использованием VarScan (2.3.7) и SNVer (0.5.3) во избежание ложноположительных результатов в процессе секвенирования, и вариации аннотировали с помощью контрольной последовательности. Большие вставки и делеции идентифицировали с использованием pindel (0.2.5a3). Некартированные прочтения собирали *de novo* с использованием SOAPdenovo (2.04). Секвенированные фрагменты повторно собирали в контиги, которые сравнивали в программе Blast с базой данных нуклеотидных последовательностей NCBI. Все прочтения выборки делили на подвыборки до 50% и затем собирали *de novo* с

использованием SOAPdenovo (2.04), получая объединенную версию *de novo* собранной последовательности BT2013.

**Последовательности**

SEQ ID NO: 1 (объединенная версия *de novo* собранной последовательности BT2013) –смотри электронный список последовательностей.

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**ФОРМУЛА ИЗОБРЕТЕНИЯ**

1. *Bacteroides thetaiotaomicron*, депонированная под регистрационным номером 42341 в NCIMB, или ее производное.

2. Композиция, содержащая *Bacteroides thetaiotaomicron* по п. 1.

3. Композиция по п. 2, в которой *Bacteroides thetaiotaomicron* является инкапсулированной.

4. Фармацевтическая композиция, содержащая *Bacteroides thetaiotaomicron* по п. 1 и фармацевтически приемлемый эксципиент, носитель или разбавитель.

5. Фармацевтическая композиция по п. 4, в которой указанная *Bacteroides thetaiotaomicron* является инкапсулированной.

6. Питательная добавка, содержащая *Bacteroides thetaiotaomicron* по п. 1 и приемлемый для питания эксципиент, носитель или разбавитель.

7. Композиция питательной добавки по п. 6, в которой указанная *Bacteroides thetaiotaomicron* является инкапсулированной.

8. Кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка, содержащие *Bacteroides thetaiotaomicron* по п. 1.

9. Кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка по п. 8, при этом указанный кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка являются инкапсулированными.

10. *Bacteroides thetaiotaomicron* по п. 1, композиция по п. 2 или 3, фармацевтическая композиция по п. 4 или 5, питательная добавка по п. 6 или 7 или кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка по п. 8 или 9 для применения с целью модуляции воспаления ткани или органа у субъекта.

11. *Bacteroides thetaiotaomicron*, композиция, фармацевтическая композиция, питательная добавка, кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка для применения по п. 10, при этом указанные *Bacteroides thetaiotaomicron*, композиция, фармацевтическая

композиция, питательная добавка, кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка способствуют уменьшению воспаления ткани или органа.

12. *Bacteroides thetaiotaomicron*, композиция, фармацевтическая композиция, питательная добавка, кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка для применения по п. 10 или 11, при этом указанная *Bacteroides thetaiotaomicron* способствует уменьшению воспаления эпителиальных клеток ткани или органа.

13. *Bacteroides thetaiotaomicron*, композиция, фармацевтическая композиция, питательная добавка, кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка для применения по п. 12, при этом указанные эпителиальные клетки представляют собой эпителиальные клетки пищеварительного тракта.

14. *Bacteroides thetaiotaomicron* по п. 1, композиция по п. 2 или 3, фармацевтическая композиция по п. 4 или 5, питательная добавка по п. 6 или 7, или кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка по п. 8 или 9 для применения в лечении и/или предотвращении заболевания у субъекта; при этом указанное заболевание представляет собой воспалительное заболевание и/или аутоиммунное заболевание, и/или аллергическое заболевание.

15. *Bacteroides thetaiotaomicron*, композиция, фармацевтическая композиция, питательная добавка, кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка для применения по п. 14, при этом указанное заболевание поражает пищеварительный тракт, секцию пищеварительного тракта и/или эпителиальные клетки.

16. *Bacteroides thetaiotaomicron*, композиция, фармацевтическая композиция, питательная добавка, кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка для применения по п. 14 или 15, при этом указанное заболевание выбирают из группы, состоящей из воспалительного заболевания кишечника (IBD), колита, ревматоидного артрита, псориаза, рассеянного склероза, диабета I

типа, целиакии, атопического дерматита, ринита, синдрома раздраженной кишки (IBS), язвенного колита, паучита, болезни Крона, функциональной диспепсии, атопических заболеваний, некротизирующего энтероколита, болезни неалкогольной жировой инфильтрации печени, желудочно-кишечной инфекции, а также их сочетаний.

17. *Bacteroides thetaiotaomicron*, композиция, фармацевтическая композиция, питательная добавка, кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка для применения по п. 14 или 15, при этом указанное заболевание представляет собой воспалительное заболевание кишечника (IBD), болезнь Крона, целиакию или язвенный колит.

18. *Bacteroides thetaiotaomicron* по п. 1, композиция по п. 2 или 3, фармацевтическая композиция по п. 4 или 5, питательная добавка по п. 6 или 7, или кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка по п. 8 или 9 для применения с целью уменьшения разрыва толстого кишечника у субъекта, предпочтительно указанный субъект имеет IBD.

19. *Bacteroides thetaiotaomicron*, композиция, фармацевтическая композиция, питательная добавка, кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка для применения по п. 18, при этом указанные *Bacteroides thetaiotaomicron*, композиция, фармацевтическая композиция, питательная добавка, кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка способствуют уменьшению или предотвращению нарушения целостности эпителия слизистой оболочки и/или уменьшению или предотвращению сокращения количества бокаловидных клеток в эпителии, и/или уменьшению или предотвращению инфильтрации иммунных клеток в собственную пластинку слизистой оболочки кишечника.

20. *Bacteroides thetaiotaomicron* по п. 1, композиция по п. 2 или 3, фармацевтическая композиция по п. 4 или 5, питательная добавка по п. 6 или 7, или кормовой продукт, пищевой продукт,

биологически активная пищевая добавка или пищевая добавка по п. 8 или 9 для применения с целью уменьшения экспрессии одного или более провоспалительных генов в клетке или клетках у субъекта.

21. *Bacteroides thetaiotaomicron*, композиция, фармацевтическая композиция, питательная добавка, кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка для применения по п. 20, при этом указанные провоспалительные гены выбирают из группы, состоящей из IL1- $\beta$ , IL6, IL8, IL10, а также их сочетаний.

22. *Bacteroides thetaiotaomicron*, композиция, фармацевтическая композиция, питательная добавка, кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка для применения по п. 20 или 21, при этом указанная клетка представляет собой клетку пищеварительного тракта (такую как клетка восходящего отдела толстого кишечника) или эпителиальную клетку (такую как эпителиальная клетка кишечника).

23. *Bacteroides thetaiotaomicron* по п. 1, композиция по п. 2 или 3, фармацевтическая композиция по п. 4 или 5, питательная добавка по п. 6 или 7, или кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка по п. 8 или 9 для применения с целью увеличения процентного содержания регуляторных Т-клеток (Treg) в пищеварительном тракте или секции пищеварительного тракта.

24. *Bacteroides thetaiotaomicron*, композиция, фармацевтическая композиция, питательная добавка, кормовой продукт, пищевой продукт, биологически активная пищевая добавка или пищевая добавка для применения по п. 23, при этом указанная секция пищеварительного тракта представляет собой собственную пластинку слизистой оболочки тонкого кишечника.

25. Способ получения фармацевтической композиции по п. 4 или 5, включающий смешивание указанной *Bacteroides thetaiotaomicron* с фармацевтически приемлемым эксципиентом, носителем или разбавителем, при этом указанная *Bacteroides thetaiotaomicron*, необязательно, инкапсулируется во время

указанного процесса.

26. Способ получения питательной добавки по п. 6 или 7, включающий смешивание указанной *Bacteroides thetaiotaomicron* с приемлемым для питания эксципиентом, носителем или разбавителем, при этом указанная *Bacteroides thetaiotaomicron*, необязательно, инкапсулируется во время указанного процесса.

27. Способ получения кормового продукта, пищевого продукта, биологически активной пищевой добавки или пищевой добавки по п. 8 или 9, включающий смешивание указанной *Bacteroides thetaiotaomicron* с кормовым продуктом, пищевым продуктом, биологически активной пищевой добавкой, пищевой добавкой или их ингредиентом; при этом указанная *Bacteroides thetaiotaomicron*, необязательно, инкапсулируется во время указанного процесса.

28. Способ модуляции воспаления ткани или органа у субъекта, включающий введение субъекту *Bacteroides thetaiotaomicron* по п. 1, композиции по п. 2 или 3, фармацевтической композиции по п. 4 или 5, питательной добавки по п. 6 или 7, или кормового продукта, пищевого продукта, биологически активной пищевой добавки или пищевой добавки по п. 8 или 9.

29. Способ лечения и/или предотвращения воспалительного заболевания и/или аутоиммунного заболевания у субъекта, включающий введение субъекту *Bacteroides thetaiotaomicron* по п. 1, композиции по п. 2 или 3, фармацевтической композиции по п. 4 или 5, питательной добавки по п. 6 или 7, или кормового продукта, пищевого продукта, биологически активной пищевой добавки или пищевой добавки по п. 8 или 9.

30. Способ уменьшения разрыва толстого кишечника у субъекта, включающий введение субъекту *Bacteroides thetaiotaomicron* по п. 1, композиции по п. 2 или 3, фармацевтической композиции по п. 4 или 5, питательной добавки по п. 6 или 7, или кормового продукта, пищевого продукта, биологически активной пищевой добавки или пищевой добавки по п. 8 или 9, предпочтительно, в случае, когда субъект имеет IBD.

31. Способ уменьшения экспрессии одного или более провоспалительных генов в клетке или клетках у субъекта,

включающий введение субъекту *Bacteroides thetaiotaomicron* по п. 1, композиции по п. 2 или 3, фармацевтической композиции по п. 4 или 5, питательной добавки по п. 6 или 7, или кормового продукта, пищевого продукта, биологически активной пищевой добавки или пищевой добавки по п. 8 или 9.

32. Способ увеличения процентного содержания регуляторных Т-клеток (Treg) в пищеварительном тракте или секции пищеварительного тракта, включающий введение субъекту *Bacteroides thetaiotaomicron* по п. 1, композиции по п. 2 или 3, фармацевтической композиции по п. 4 или 5, питательной добавки по п. 6 или 7, или кормового продукта, пищевого продукта, биологически активной пищевой добавки или пищевой добавки по п. 8 или 9.

33. Применение *Bacteroides thetaiotaomicron* по п. 1, композиции по п. 2 или 3, фармацевтической композиции по п. 4 или 5, питательной добавки по п. 6 или 7, или кормового продукта, пищевого продукта, биологически активной пищевой добавки или пищевой добавки по п. 8 или 9 в производстве лекарственного средства для модуляции воспаления ткани или органа у субъекта.

34. Применение *Bacteroides thetaiotaomicron* по п. 1, композиции по п. 2 или 3, фармацевтической композиции по п. 4 или 5, питательной добавки по п. 6 или 7, или кормового продукта, пищевого продукта, биологически активной пищевой добавки или пищевой добавки по п. 8 или 9 в производстве лекарственного средства для лечения и/или предотвращения воспалительного заболевания и/или аллергического заболевания, и/или аутоиммунного заболевания у субъекта.

35. Применение *Bacteroides thetaiotaomicron* по п. 1, композиции по п. 2 или 3, фармацевтической композиции по п. 4 или 5, питательной добавки по п. 6 или 7, или кормового продукта, пищевого продукта, биологически активной пищевой добавки или пищевой добавки по п. 8 или 9 в производстве лекарственного средства для уменьшения разрыва толстого кишечника у субъекта, предпочтительно в случае, когда субъект имеет IBD.

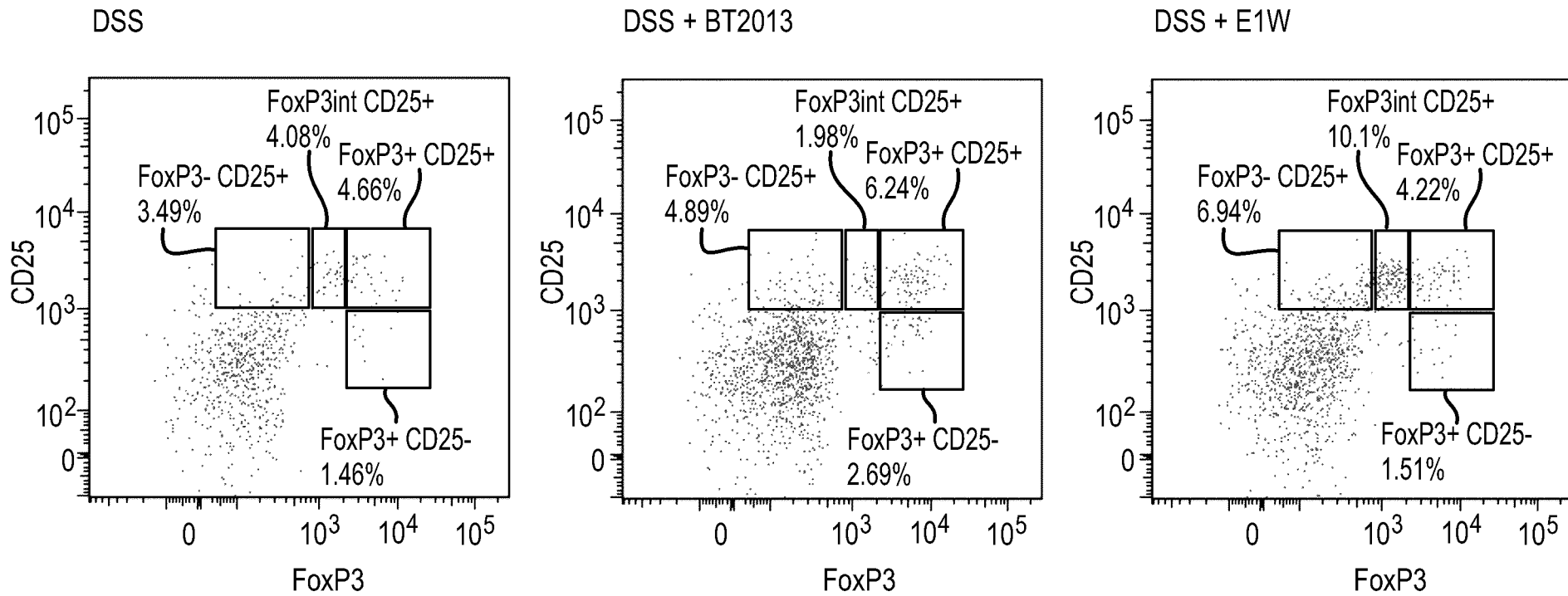


36. Применение *Bacteroides thetaiotaomicron* по п. 1, композиции по п. 2 или 3, фармацевтической композиции по п. 4 или 5, питательной добавки по п. 6 или 7, или кормового продукта, пищевого продукта, биологически активной пищевой добавки или пищевой добавки по п. 8 или 9 в производстве лекарственного средства для уменьшения экспрессии одного или более провоспалительных генов в клетке или клетках у субъекта.

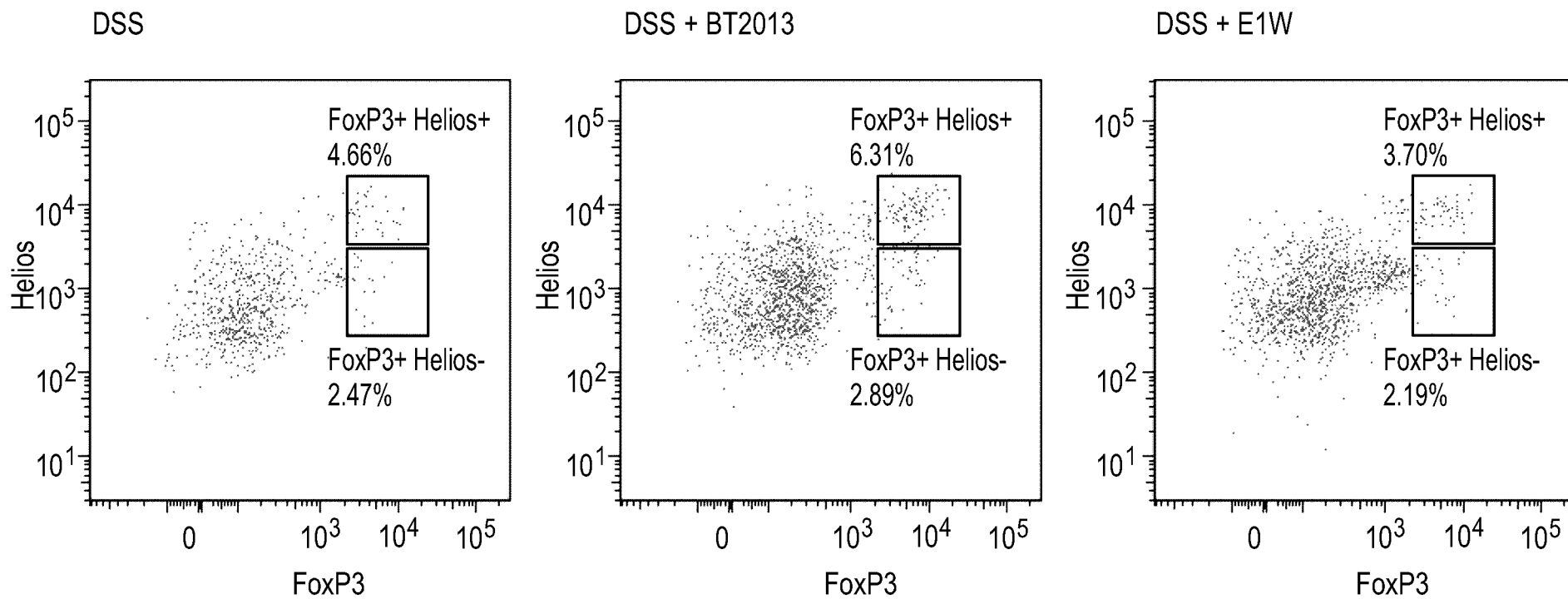
37. Применение *Bacteroides thetaiotaomicron* по п. 1, композиции по п. 2 или 3, фармацевтической композиции по п. 4 или 5, питательной добавки по п. 6 или 7, или кормового продукта, пищевого продукта, биологически активной пищевой добавки или пищевой добавки по п. 8 или 9 в производстве лекарственного средства для увеличения процентного содержания регуляторных Т-клеток (Treg) в пищеварительном тракте или секции пищеварительного тракта.

По доверенности

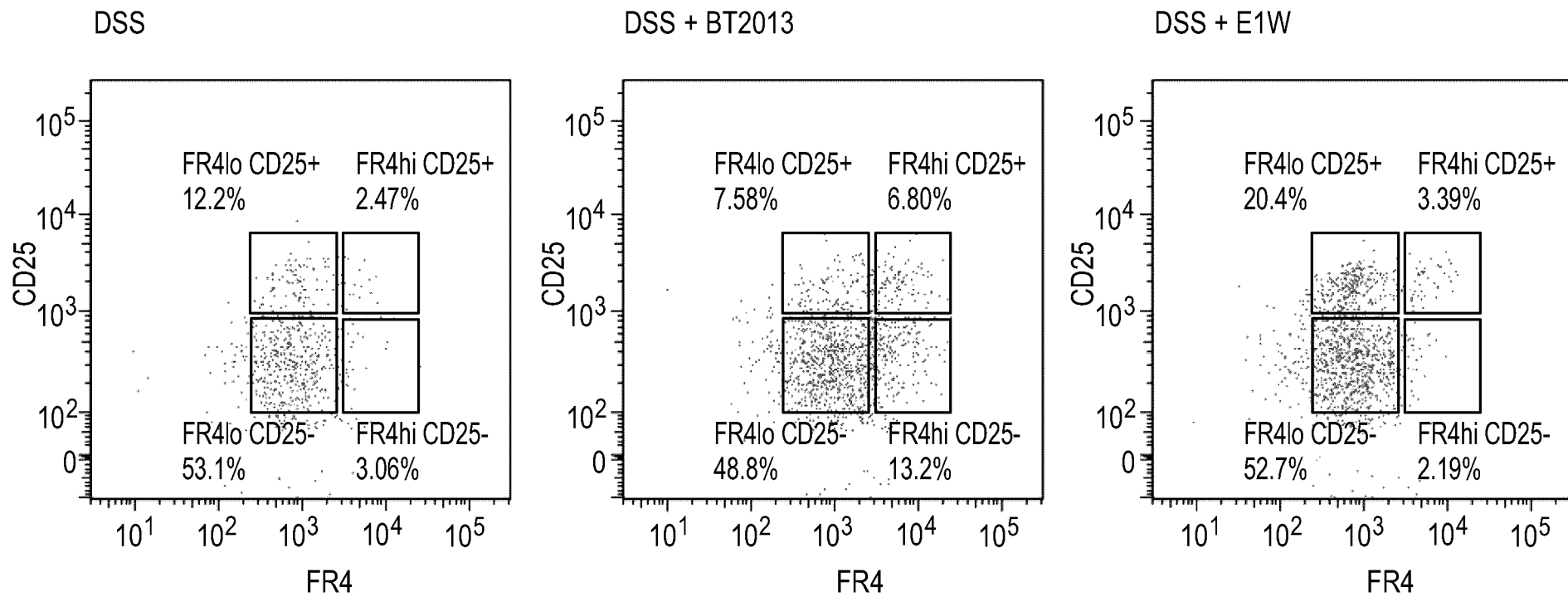
ФИГ. 1



ФИГ. 1 (продолжение)

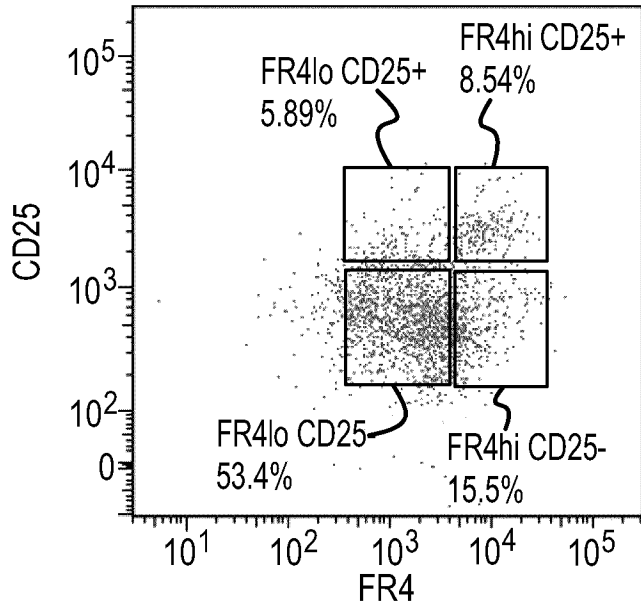
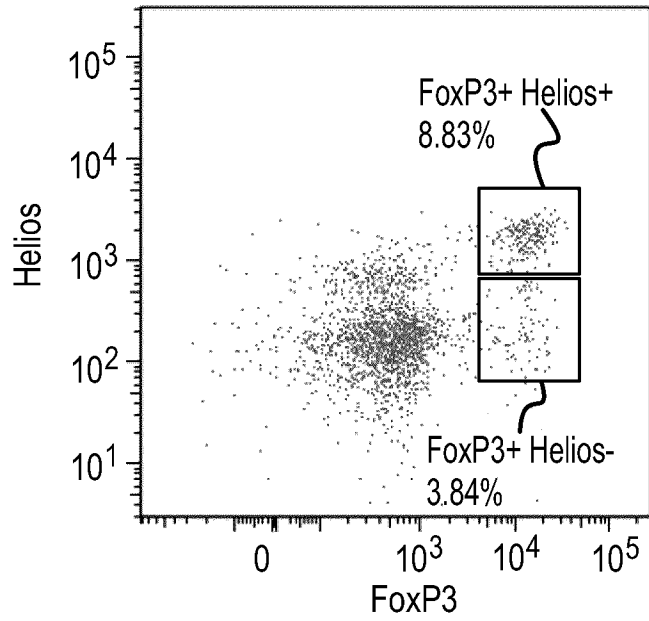
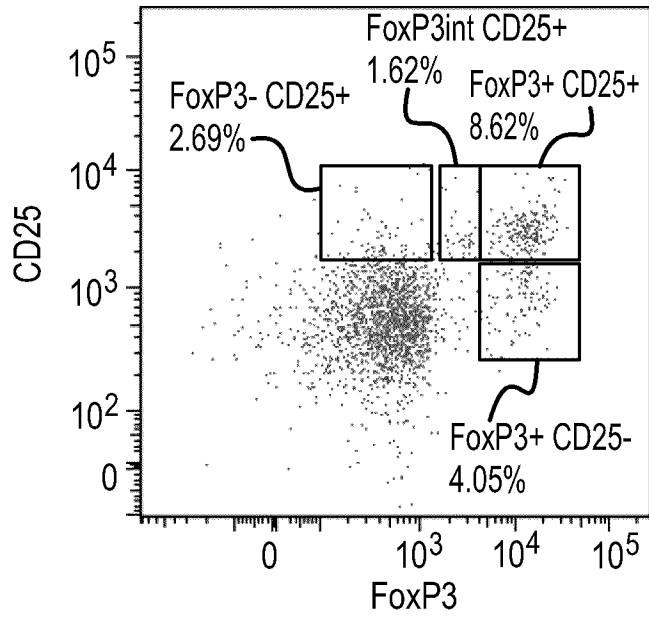


ФИГ. 1 (продолжение)

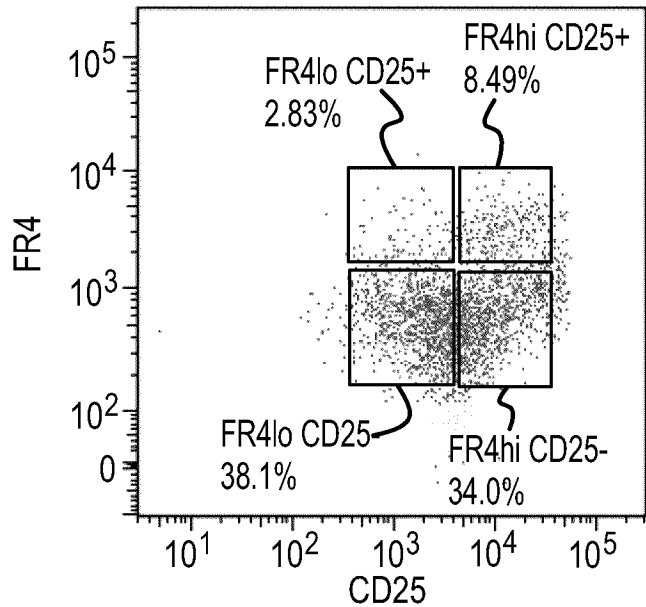
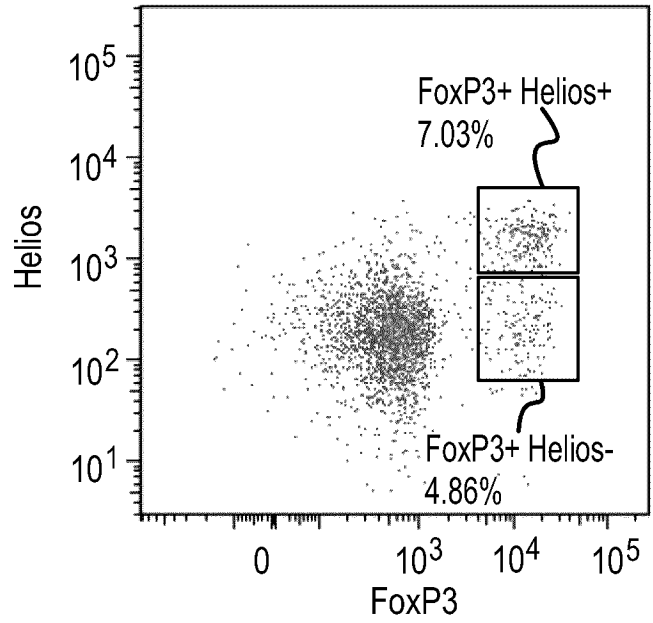
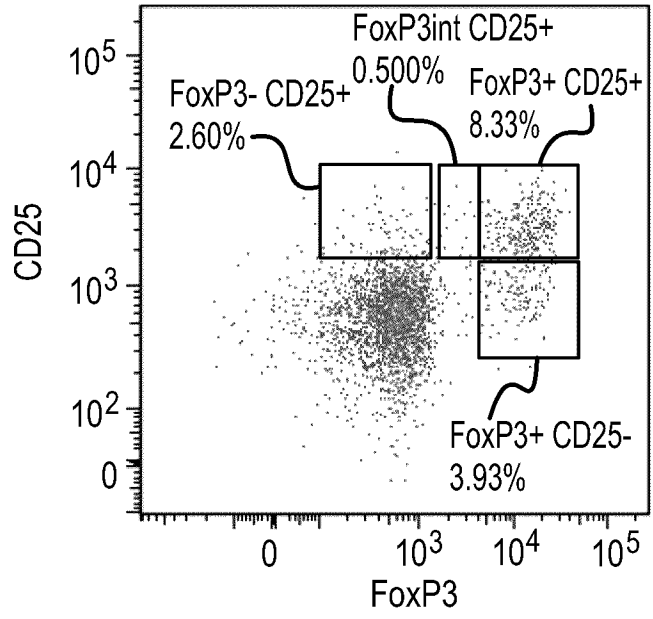


НЕ ПОДВЕРГНУТЫЕ ВОЗДЕЙСТВИЮ

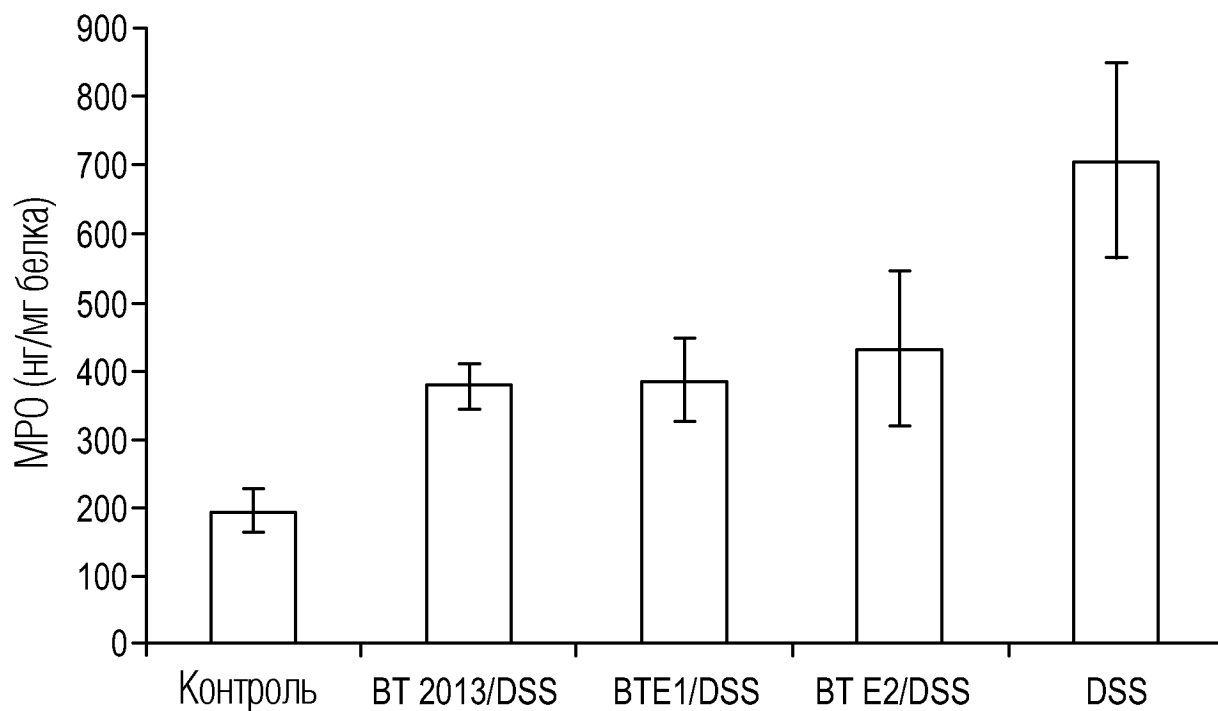
ФИГ. 2



ФИГ. 2 (продолжение)

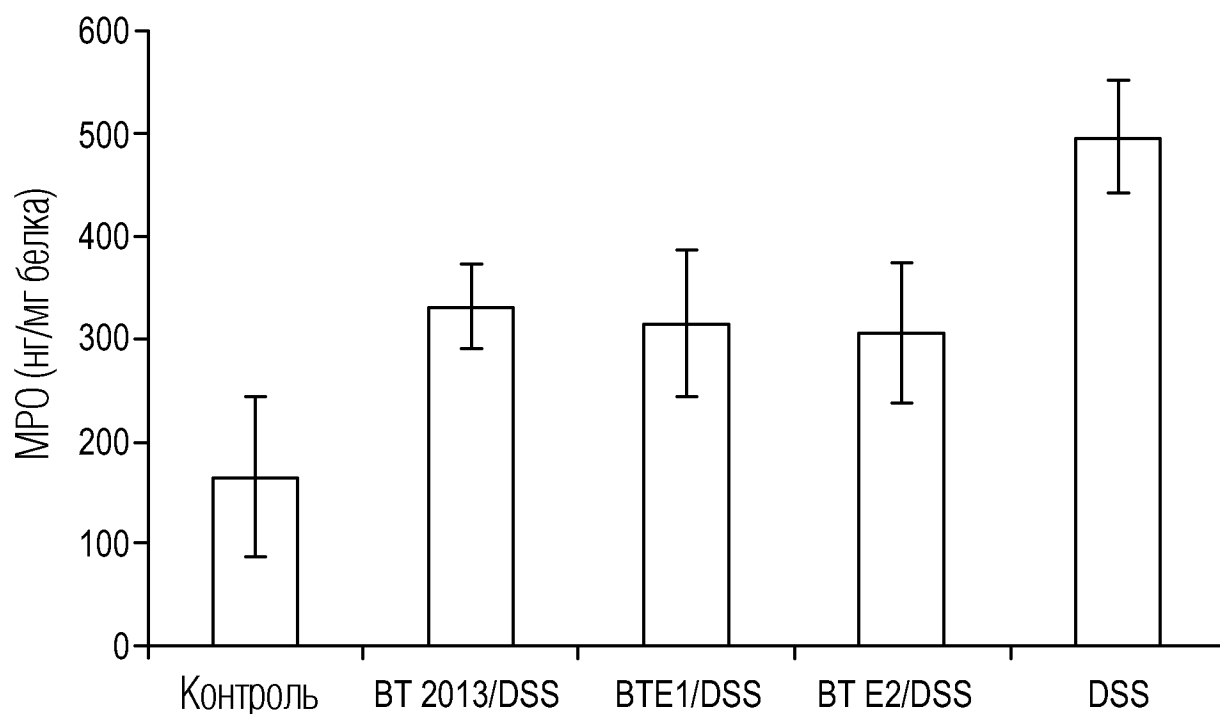


ФИГ. 3а



контроль против BT2013	*	P<0.05
контроль против BT E1	*	P<0.05
контроль против BTE2	**	P<0.01
контроль против DSS	***	P<0.001
BT2013 против BTE1	н/з	P>0.05
BT2013 против BTE2	н/з	P>0.05
BT2013 против DSS	***	P<0.001
BT E1 против BTE2	н/з	P>0.05
BT E1 против DSS	***	P<0.001
BTE2 против DSS	***	P<0.001

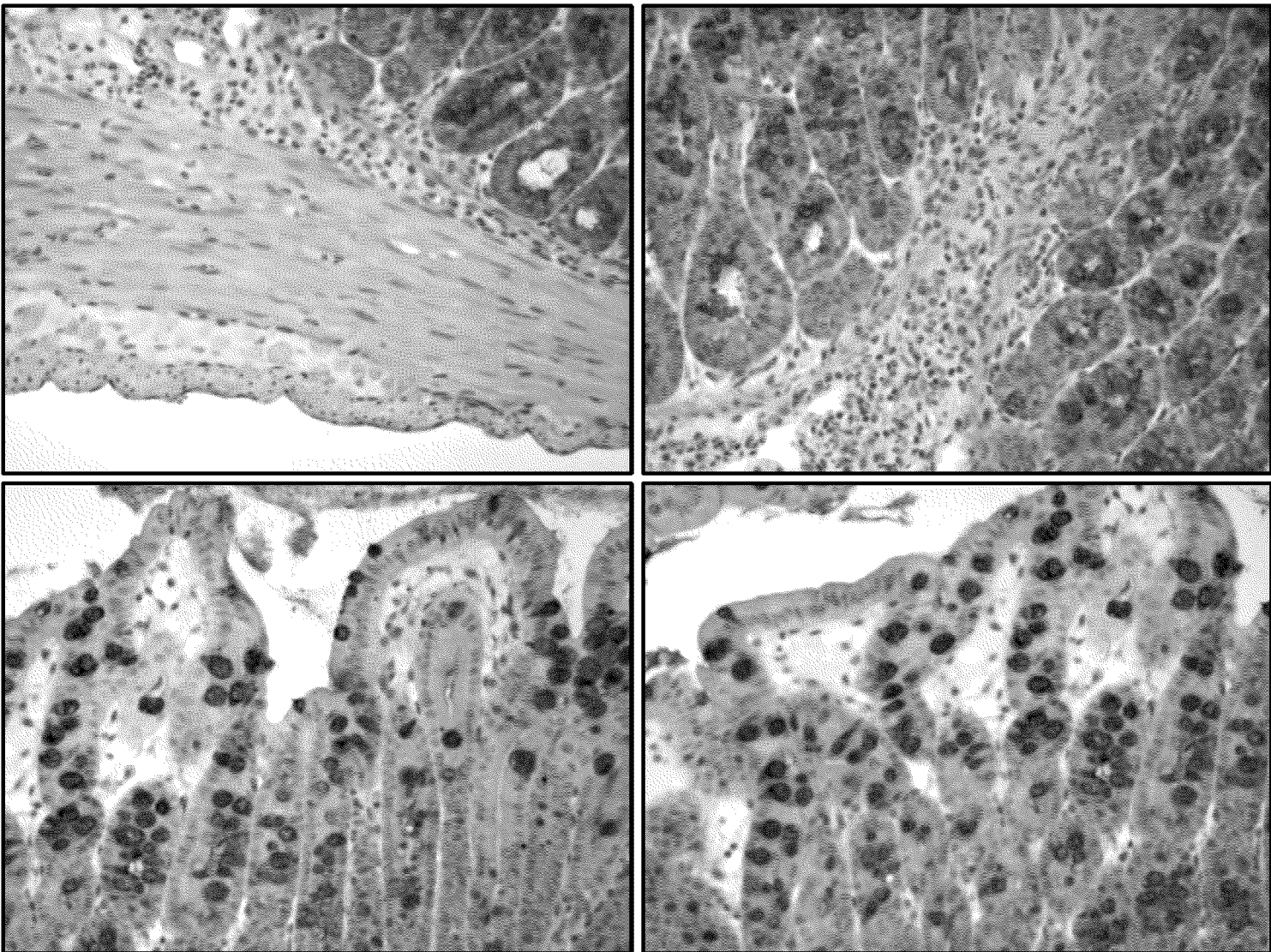
ФИГ. 3b



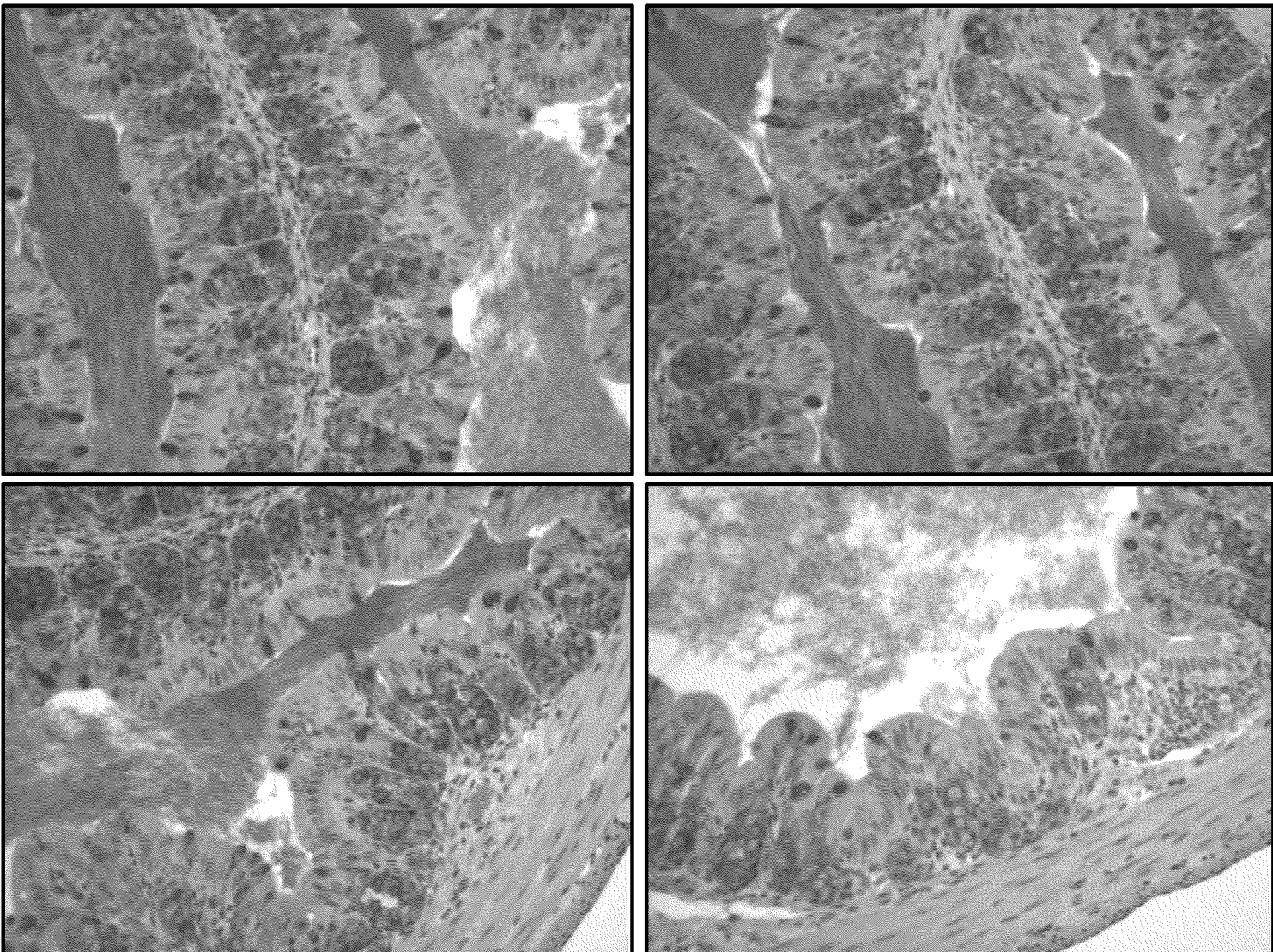
контроль против BT2013	**	P<0.01
контроль против BT E1	*	P<0.05
контроль против BTE2	*	P<0.05
контроль против DSS	***	P<0.001
BT2013 против BTE1	н/з	P>0.05
BT2013 против BTE2	н/з	P>0.05
BT2013 против DSS	**	P<0.01
BT E1 против BTE2	н/з	P>0.05
BT E1 против DSS	**	P<0.01
BTE2 против DSS	**	P<0.01



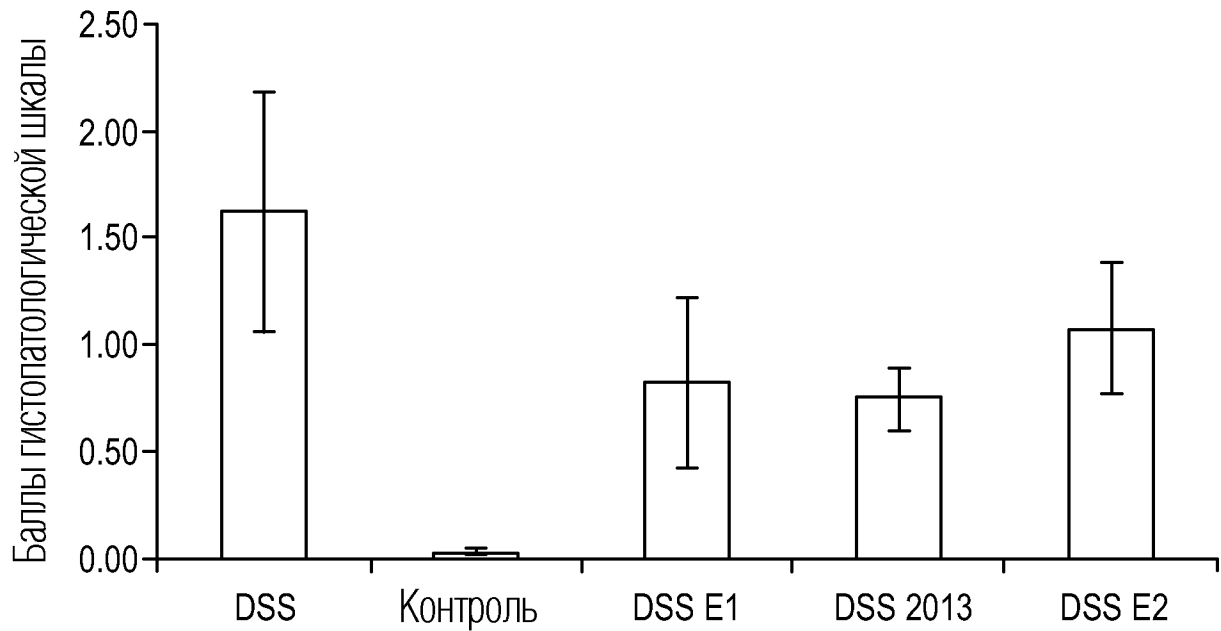
ФИГ. 4а



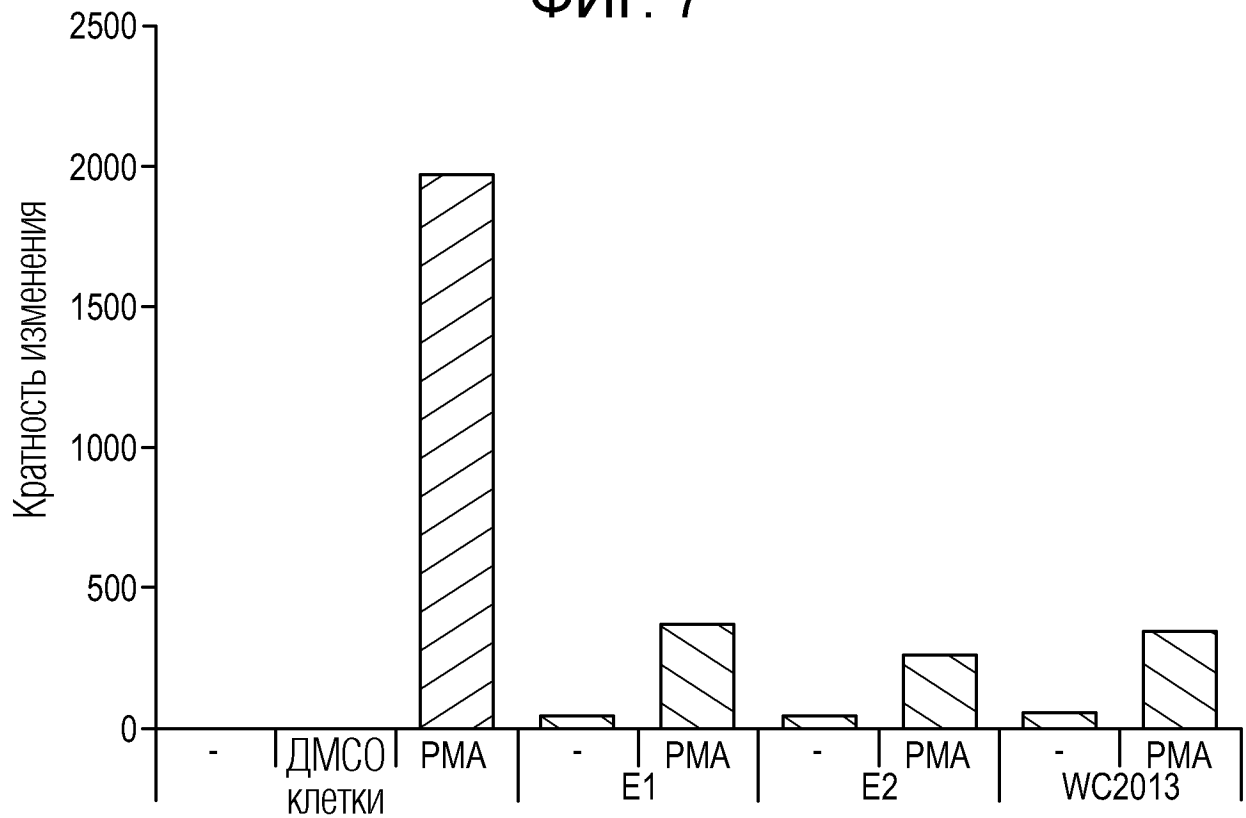
ФИГ. 4b



ФИГ. 5



ФИГ. 7



ФИГ. 6

