NatureCleanSkin

		Slide names
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Areas of Medicine	Dermatology, Immunology, Gastroenterology, Microbiology, Bioinformatics	
Date	July 1, 2018	
(A) Working Title	Scientific research "**Development of new methods of complex diagnostics of metagenomes* and microbiomes of throat, intestine, blood and skin of psoriatic patients, and also their corrections for achievement of long and steady remission**". Total researh consists of two consecutive stages: Stage 1 (NIR1). Metagenomes of whole blood and skin phagocytes at psoriatic disease. Stage 2 (NIR2). Metagenomes of whole blood, metagenomes and microbiomes of throat and gastrointestinal lavage** water and permeability of small intestine at psoriatic disease. Development and approbation of new technique for treatment of psoriatic disease based on correction of throat and/or gastrointestinal microbiomes.	Presentation (english version)
	* Metagenome is a complex of all nhDNA (non-host DNA, that is, non-human here) contained in a biomaterial. nhDNA is a bacterial, archean, fungal, helminthic, viral, phage, etc. DNA. ** Gastrointestinal lavage water - a biomaterial collected closer to end of Intestinal lavage or Prakshalana.	

(B) Main questions	**Question 1.** Does severity of psoriatic disease correlate a) with concentration of any nhDNA in whole blood? b) with level of PAMP-nemia***? **Question 2.** Does nondegradeted nhDNA come from blood into psoriatic skin? If so, which part of whole blood metagenome is found in metagenome of phagocytes of psoriatic skin and in what concentration? **Question 3.** Specific changes in the parietal intestinal microbiome and increased permeability of small intestine are the main causes of excess intake of specific bacterial products in blood in psoriatic disease? **Question 4.** Does stable correction of parietal small intestinal microbiome lead to a long-term remission of psoriatic disease?	Stage1-Q1 Stage1-Q2 Stage1-Q1&2 Stage2-MQ Stage2-Q3 Stage2-Q4
	*** PAMP-nemia is an increased kPAMP-load on blood phagocytes and elevated level (concentration) of kPAMP in blood. The main kPAMP (key PAMP) is PG - peptidoglycan and LPS - lipopolysaccharide.	
(C) Basic publica-tions	Baker BS, Powles A, Fry L. Peptidoglycan: a major aetiological factor for psoriasis? Trends Immunol. 2006 Dec;27(12):545-51. 17045843.	
	Fahlen A, Engstrand L, Baker BS, Powles A, Fry L. Comparison of bacterial microbiota in skin biopsies from normal and psoriatic skin. Arch Dermatol Res. 2012 Jan;304(1):15-22. 22065152.	
	Fry L. Microbiome of chronic plague psoriasis. Chapter 21 in The Human Microbiota and Chronic Disease: Dysbiosis as a Cause of Human Pathology. 2016, John Wiley & Sons, Inc. ISBN 9781118982877, link.	
	Grumaz S, Stevens P, Grumaz C. et al. Next-generation sequencing diagnostics of bacteremia in septic patients. Genome Med. 2016 Jul 1;8(1):73. 27368373.	
	Gyarmati P, Kjellander C, Aust C. et al. Metagenomic analysis of bloodstream infections in patients with acute leukemia and therapy-induced neutropenia. Sci Rep. 2016 Mar 21;6:23532. 26996149.	
	Li Q, Wang C, Tang C, Zhao X, He Q, Li J. Identification and Characterization of Blood and Neutrophil-Associated Microbiomes in Patients with Severe Acute Pancreatitis Using Next-Generation Sequencing. Front Cell Infect Microbiol. 2018 Jan 23;8:5. 29423379.	
	Nakatsuji T, Chiang HI, Jiang SB The microbiome extends to subepidermal compartments of normal skin. Nat Commun. 2013;4:1431. 23385576.	
	Païssé S, Valle C, Servant F. et al. Comprehensive description of blood microbiome from healthy donors assessed by 16S targeted metagenomic sequencing. Transfusion. 2016 May;56(5):1138-47. 26865079.	
	Peslyak MY, Gumayunova NG, Nesterov AS, Potaturkina-Nesterova NI, Small intestine microflora at psoriasis. Its possible role in pathogenesis, Abstracts of the 3rd World Psoriasis & Psoriatic Arthritis Conference 2012, Stockholm, Dermatol Ther 2012, 2(10), S12. link, link-R.	
	Yun Longa, Yinxin Zhangb, Yanping Gongb et al. Diagnosis of Sepsis with Cell-free DNA by Next-Generation Sequencing Technology in ICU Patients. Arch Med Res. 2016 Jul;47(5):365-371. 27751370.	
	Гумаюнова Н.Г. Синдром избыточного роста бактерий в тонкой кишке при псориатической болезни на фоне бластоцистной инвазии. дис. кмн, Челябинск, 2009, 169 с., link. Gumayunova NG. Syndrome of small intestine bacterial overgrowth at psoriatic disease against blastocystic invasion. Dissertation, Chelyabinsk, 2009, 169 p, (Rus), link.	
	Короткий Н.Г., Песляк М.Ю. Методика лечения хронических кожных болезней (очищение, диета, микрофлора).	

	Лечебное дело, 2006, 4, с.37-41. link1, link2, elib. Korotky NG, Peslyak MY. The treatment of chronic skin diseases (cleansing, diet, microflora). Medical work, 2006, 4, p.37-41.	
	Короткий Н.Г., Песляк М.Ю. Псориаз как следствие включения beta-стрептококков в микробиоценоз кишечника с повышенной проницаемостью (концепция патогенеза). Вестник дерматологии и венерологии, 2005;(1): 9-18, link1, link2, elib Korotky NG, Peslyak MY. Psoriasis as a consequence of incorporation of beta-streptococci into the microbiocenosis of highly permeable intestines (a pathogenic concept), Vestn Dermatol Venerol, 2005;(1): 9-18. (Rus), ISSN 0042-4609, link.	
	Медицинская токсикология. Национальное руководство. Под ред. Е.А.Лужникова, Гэотар-медиа, 2012, 928 с., ISBN 9785970429716. Раздел 4.2.1. Кишечный лаваж, Маткевич В.А. (с.162-185). link. Medical toxicology. National leadership. Ed. Luzhnikov EA, Geotard-Media, 2012, 928 pp., ISBN 9785970429716. Section 4.2.1. Intestinal lavage, Matkevich VA (p.162-185).	
	Пегано Д. Лечение псориаза – естественный путь. "Кудиц-пресс", 2009; 264 с. ISBN 9785911360627. Pagano J. Healing psoriasis: The natural alternative., 2008, 352 p, ISBN: 9780470267264	
	Песляк М.Ю. Модель патогенеза псориаза. Часть 1. Системный псориатический процесс, Москва, MYPE, 2012, 94 с, ISBN 9785905504013, link. Peslyak MY. Model of pathogenesis of psoriasis. Part 1. Systemic psoriatic process, Moscow, MYPE, 2012, 84 p., ISBN 9785905504020, link.	
	Песляк М.Ю. Модель патогенеза псориаза. Часть 2. Локальные процессы, Москва, MYPE, 2012, 116 с, ISBN 9785905504037, link Peslyak MY. Model of pathogenesis of psoriasis. Part 2. Local processes. Moscow, MYPE, 2012, 110 p., ISBN 9785905504044, link.	
(D) Motivation/ Problems/ Purposes.	**Motivation.** The Psoriatic Disease (PD) takes place at 2-3% of the population of Earth, annually for the first time get sick with psoriatic disease of 4-6 million people. The disease lasts during all subsequent life of the patient, is spontaneous or thanks to treatment being weakened, and then again recurs. The key causes, supports and aggravation of psoriatic disease are unknown, the uniform conventional model of pathogenesis does not exist.	Psoriatic_disease Patient_Stat-C Patient_Stat-R
	Problems. • The majority of offered models of pathogenesis of PD are local (not systemic) and assume that the main reasons for psoriatic rashes are directly in skin (Peslyak 2012_2). • The existing methods of treatment of PD, are based on local models of pathogenesis, directed to decrease of manifestations of PD, have collateral adverse effects. They (especially with use of biologics) have high price, do not guarantee long remission and have lowered efficiency at repeated use.	Video Antigen-1 Antigen-2 Antigen-3

	Purposes: • Studying of the subprocesses which are the cornerstone of systemic psoriatic process. • Confirmation of basic hypotheses of the system model of pathogenesis of PD based on key role of parietal microbiome and macromolecular permeability of small bowel and income of specific bacterial products from system blood flow in psoriatic derma. • Development and use of the technique of treatment of PD based on stable correction of parietal microbiome and permeability of small bowel for the purpose of achievement of long-term remission.	
THREE	**Three core aspects (Ideas/hypoteses + Data + Tools)**	
(E) **Ideas and hypoteses**	**Main ideas:** • Chronization of inflammatory processes in psoriatic skin is caused by constant income from blood flow of tolerized phagocytes containing endocytosed in blood (but not degraded) nonhost biomaterial, in particular nhDNA, LPS, PG (including PG-Y). • Long-term remission of PD can be reached thanks to stable full (almost full) elimination pathogenic and presumed psoragenic PsB bacteria from parietal small intestine microbiome and normalization of macromolecular permeability of small intestine.	
	Theoretical background: Psoriatic patients have SIBO (small intestine bacterial overgrowth). There are colonies of pathogenic and PsB bacteria - presumed psoragenic (with peptidoglycan similar to Streptococcus pyogenes peptidoglycan) (Baker 2006, Peslyak & Gumayunova 2012, Gumayunova 2009, Korotky & Peslyak 2005). Increased macromolecular permeability of small intestine allows chronically excess income of bacterial products in blood flow and, in particular, such PAMP as PG (peptidoglycan) and LPS (endotoxin = lipopolysaccharide). PG and LPS synergic affect blood phagocytes, activating them. Part of activated phagocytes is tolerized and forms fraction of tolerized blood phagocytes. In tolerized phagocytes level of proteins responsible for degradation of endocytosed bacterial products is lowered therefore they became carriers of antigene material. Set of listed subprocesses leading to appearance in blood flow of fraction of tolerized phagocytes - carriers of specific antigene material, is called **systemic psoriatic process SPP**. Tolerized blood phagocytes (monocytes Mo-T and dendritic cells DC-T) get chemostatus similar not activated and therefore can be attracted to places of inflammations in any tissue and, in particular, in derma. These monocytes and dendritic cells, being attracted in inflamed derma, under influence of cytokines will	

	be reprogrammed and transform to mature dendritic cells. Some of them (monocytes Mo-R and dendritic cells DC-R) contain Y-antigens. Mature dendritic cells formed from Mo-R and DC-R will present Y-antigens to specific T-lymphocytes. Skin immune system can incorrectly interpret Y-antigens presentation as a sign of external PsB-infection and switch one of mechanisms of protection against bacterial infection - epidermal hyperproliferation. Detailed justification and description of systemic Y-model of pathogenesis of psoriasis, and also review of other models of pathogenesis contains in (Peslyak 2012_1, Peslyak 2012_2).	
	Hypotheses which are cornerstone of systemic psoriatic process SPP which are supposed to be checked (what is supposed to be checked is allocated): **Hypothesis H1**. Two main reasons of SPP Subprocess SP1. Increased macromolecular permeability of small intestine. Subprocess SP2. Inclusion in parietal small intestine colonies of Gram+ NOD2-active (including PsB) and Gram(-) TLR4-active bacteria. **Hypothesis H2.** PsB is Enterococcus faecalis, beta-hemolitic streptococci, VGS (Viridans group streptococci) and some other (almost complete list can be determined by KEGG database (kegg.jp). All PsB have PG-Y - peptidoglycan with interpeptide IB-Y bridges, i.e. (L-Ala)-(L-Ala) and/or (L-Ser)-(L-Ala) (i.e. similar to Streptococcus pyogenes peptidoglycan). **Hypothesis H3.** PAMP-nemia (endotoxinemia (LPS-nemia) + PG-nemia) and (PG-Y)-nemia - is the main subprocesses of SPP. defining part of PAMP-load is created by LPS from Gram(-) TLR4-active and PG from Gram+ NOD2-active bacteria. **Hypothesis H10-S (simplified H10).** Nondegraded nonhost biomaterial moves to psoriatic skin within blood phagocytes.	PG_PsB-1 PG_PsB-3
(F) Data	**(F1)** *What data do you propose to use:* Results of polls, consultations and inspections PP (psoriatic patients) and HP (healthy persons). *Place of implementation of project in Russia:* Moscow *Countries, region from which patients will be attracted:* Russia, Moscow region (possibly other). *Why:* Duration of project makes several years, PP during all project have to undergo inspections and get advice. Also from results of WMS tests of fecal microbiome it is known that its microbial composition significantly depends on region of residence. *Who will be attracted in project:* PP and HP meeting certain requirements. *Project duration:* 3 years: Stage 1 (NIR1) - 1 year, Stage 2 (NIR2) - 2 years. *As it is often supposed to obtain data*: Constantly during all project.	

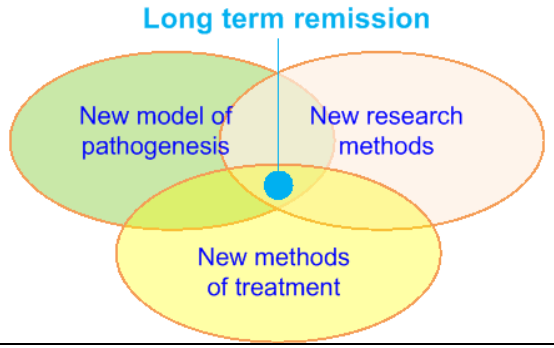
	Types of data: non-numerical and numerical (continuous and category).	
	(F2) S*ample size (Russia):* Stage 1 (NIR1): 30 PP, 10 HP Stage 2 (NIR2): 68 PP (stages 2-1 and 2-2), 40 PP (stages 2-3, 2-4, 2-5) After stage 2-2 from 68 PP will remain only 40 PP which are most meeting requirements for participation in Stages 2-3, 2-4 and 2-5.	
	(**F3)** *Data sources:* Results of inspections and tests (executed prior to beginning of project and during project), questioning. *Data already exist and are available:* partly. *Data will be obtained within project:* yes, generally. *Manual (viewing of sources, medical records, etc.) data collection will be required:* no. *Data will be obtained by poll (questioning):* yes, partly. *Data will be obtained by tests (measurements) with use of equipment:* yes, generally.	
	(F4) *Problems with lack of data: no* *Problems of collecting and data storage:* It is supposed to get and/or develop specialized software for cloudy storage of medical cards. There are problems of combination of data obtained in various medical centers. *Problems of processing (adjustment) of data:* So far not.	
	(F5) *Obstacles for data acquisition:* no. *Reliability (compliance of test results to what was supposed to be defined):* • Interpretation of results of WMS (Whole metagenome sequencing). • Interpretation of results lavage SIBO-test (in what proportion they contain information about parietal small intestine microbiome? Qualitative or quantitative?) • How precisely PP will keep diaries, independently controlling observance of personal regime and health (in particular PASI)? *Reliability (lack of contradictions):* yes. *Importance for conclusions: no*	
(G) Tools	**(G1)** *Design of project:* **Stage 1 (NIR1).** **Stage 1-1.** Selection and preparation. **Stage 1-2.** Definition of whole blood metagenomes. Definition and studying of PAMP-nemia. **Stage 1-3.** Definition of metagenomes of phagocytes of psoriatic skin. Complex studying of metagenomes of whole blood and phagocytes of psoriatic skin (only for PP).	NCS Stages1&2 Stage1 Stage1-Order Stage2 Stage2-Order

	Stage 2 (NIR2). **Stage 2-1.** Selection and preparation. **Stage 2-2 (diagnostic):** Observation analytical cross-sectional study As risk factors are considered: Existence of specific bacterial products in blood. Their range is similar to range of nonhost DNA, information on which will be obtained by WMS tests of whole blood and phagocytes of psoriatic skin (Fahlen 2012, Gyarmati 2016, Nakatsuji 2013). Macromolecular permeability of small intestine, presence of specific bacteria as a part of parietal small intestine microbiome and/or throat microbiome (independent cultural and WMS diagnostics). **Stages 2-3 and 2-4 (medical) and 2-5 (control)**: Experimental nonrandomized uncontrollable study in one group PP. Use only of such medicines which have registration is supposed. **SPT** - Stage of preliminary treatment - for PP which have chance in 2-4 months to remove causes (revealed during Stage 2-2) for which they were not allowed to Stage 2-3.	Regime Stage2-com1 Stage2-com2 Stage2-Y-treat Stage2-LST Stage2-Phage
	(G2) *Whether qualitative and/or quantitative characters will be studied:* Both that and others.	
	(G3) *Necessary experts, their qualification:* Specialist in conducting clinical trials (monitor), expert certified on performance of intestine lavage, specialist in WMS, specialist in sample preparation of biomaterial to WMS-test, immunologist, otolaryngologist, dermatologist, gastroenterologist, nutritionist, microbiologist, bioinformation scientist.	
	(G4) *Necessary equipment and resources:* Medical centers having experience, conditions and equipment for performance of consultations, diagnostics and treatments according to project program.	

<table>
<tr><th>TWO</th><th>Two key questions (What's New? So What?)</th><th></th></tr>
<tr><td>(H)
What's
New?</td><td colspan="2">New idea: New systemic model of pathogenesis of PD.

New methods of research:
• Whole metagenome sequencing (WMS) of psoriatic biopsy, whole blood and gastrointestinal waters (adaptation of known methods is supposed) (Gyarmati 2016,Yun 2016).
• Lavage SIBO-test. As biomaterial containing parietal microbiome of intestine lavage waters received during intestine lavage (or Prakshalana) are used (Medical Toxicology 2012).
• Cultural and metagenomic testing of the same biomaterials (lavage waters and throat swab) will allow to compare and to mutually add results, will increase their reliability.

New technique of treatment:
The main difference of Y-technique from Pagano regime (Korotky 2006, Pagano 2009) is concluded that Y-technique includes stage of consultations and inspection on basis of which results PCT (personal course of treatment) is formed. PCT is cornerstone use of intestine lavage (or prakshalana) and phagotherapy of small intestine microbiome.

Set of these component (each of which contains novelty aspects) leads to required purpose - long-term remission.</td><td>Stage1-new

Stage2-new</td></tr>
<tr><td>(I)
So What?</td><td colspan="2">Why it is important to learn answers to the main questions:
(I1) Question 1. Whether severity of psoriatic disease correlates
a) with concentration of any nhDNA in whole blood?
b) with PAMP-nemia level ***?

Preliminary answer is supposed to be received upon termination of Stage 1-2 (NIR1), end of project in general has to buttress up (or not) its additional by facts.
Affirmative answer will mean that
• systemic Y-model of pathogenesis of PD has to be considered as partly confirmed;</td><td></td></tr>
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	• further researches are justified and necessary for check of other hypotheses on which Y-model is based; • carrying out subsequent Stages is proved; Negative answer will mean that • systemic Y-model of pathogenesis of PD needs to be corrected. • content and order of carrying out subsequent Stages have to be revised.	
	(I2) Question 2. Whether nondegraded nhDNA from blood comes to psoriatic skin? If yes, that what part of whole blood metagenome is found in metagenome of phagocytes of psoriatic skin and in what concentration? Answer is supposed to be received after Stage 1-3 (NIR1). Affirmative answer will mean that • systemic Y-model of pathogenesis of PD has to be considered as partly confirmed; • further researches are justified and necessary for check of other hypotheses on which Y-model is based; • carrying out subsequent Stages is proved; Negative answer will mean that • systemic Y-model of pathogenesis of PD needs to be corrected. • content and order of carrying out subsequent Stages have to be revised.	
	(I3) Question 3. Specific changes in parietal small intestine microbiome and hyperpermeability of small intestine - the main reasons for excess income of specific bacterial products in blood at psoriatic disease? Preliminary answer is supposed to be received after Stage 2-2, end of project in general has to buttress up (or not) its additional by facts. Affirmative answer will mean that • systemic Y-model of pathogenesis of PD has to be considered as partly confirmed; • further researches are justified and necessary for check of other hypotheses on which Y-model is based; • optimum and effective methods of treatment of PD have to rely on diagnostics and correction of parietal small intestine microbiome and macromolecular permeability of small intestine; • carrying out subsequent Stages is proved; Negative answer will mean that • systemic Y-model of pathogenesis of PD needs to be corrected. • content and order of carrying out subsequent Stages have to be revised.	

	(I4) Question 4. Whether stable correction of parietal small intestine microbiome leads to long-term remission of psoriatic disease? Answer can be received only upon termination of project in general. Affirmative answer will mean that • systemic Y-model of pathogenesis of PD has to be considered as partly confirmed; • further researches are justified and necessary for check of other hypotheses on which Y-model is based; • optimum and effective methods of treatment of PD have to rely on diagnostics and correction of parietal small intestine microbiome and macromolecular permeability of small bowel; • technique of diagnostics and treatment of PD applied in project is effective and can be recommended for broad use; Negative answer will mean that • or systemic Y-model of pathogenesis of PD is not quite correct and needs to be corrected; • or applied technique of diagnostics and treatment of PD has serious shortcomings and has to be processed; • or both of these statements are fair;	
	(I5) **What main decisions / behavior/actions, etc. will be consequence of successfully executed project?** • Publications in scientific and popular scientific press results of project, wide use of information obtained within project among experts and patients; • Creation of dermatological center (network of dermatological centers) for patients having chronic dermatosis pathogenesis (severity) of which depends on microbiome and/or permeability of intestine. • Creation of medical center (network of medical centers) of wider profile for a) patients with chronic dermatosis, including with associated diseases (according to NPF among PP in USA such 50-70%). b) patients with diseases in which pathogenesis (severity) important or crucial role is played (or it is supposed what plays) by intestine microbiome and/or permeability. • Preparation and carrying out new project(s) directed to more detailed research of system model of pathogenesis of PD, check of other hypotheses on which it is based; • On improvement of technique of treatment of PD taking into account existence at PP some associated diseases, first of all those which are risk factors of emergence and support of SIBO and/or disturbance of macromolecular permeability of small intestine;	
ONE	**One bottom line**	
(J) Scientific contribution	*What main scientific contribution of successfully executed project will be:* • Experimental proof of part of hypotheses which are cornerstone of systemic Y-model of pathogenesis of PD that will entail revolutionary changes in understanding of pathogenesis of PD; • Development and deployment of new technique of diagnostics and treatment of PD leading to long remission;	