FNSNF
SCHWEIZERISCHER NATIONALFONDS
ZUR FÖRDERUNG DER WISSENSCHAFTLICHEN FORSCHUNG

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Internationale Zusammenarbeit
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Herr Prof. Valentin Djonov
Institut für Anatomie
Medizinische Fakultät
Universität Bern
Baltzerstrasse 2
CH-3012 Bern

Bern, 26. März 2014

Verfügung
IZ73Z0_152454 / 1

Sehr geehrter Herr Prof. Djonov

Wir freuen uns, Ihnen mitzuteilen, dass der Forschungsrat beschlossen hat, für das Projekt "Role of blood flow and SD-1/CXCR4-induced recruitment of mononuclear cells in intussuceptive angiogenesis" einen Forschungsbeitrag von CHF 240'000.00 zuzusprechen. Weitere Angaben zur Beurteilung Ihres Gesuches finden Sie direkt in mySNF.

Die Aufteilung und die Bedingungen der Zusprache im Anhang bilden Bestandteil dieses Beschlusses.

Im Weiteren sind insbesondere die Bestimmungen des "Beitragsreglements" und des "Allgemeinen Ausführungsreglements zum Beitragsreglement" zu beachten. Die Reglemente sind auf dem Server des Schweizerischen Nationalfonds zugänglich (vgl. "Relevante Rechtsdokumente" im Anhang). Auf Anfrage senden wir Ihnen gerne ein Exemplar zu. Im Leitfaden *„Guidelines for the lifetime management of SCOPES Joint Research Projects and Institutional Partnerships"* finden Sie umfassende Informationen zur Beitragsabwicklung (unter www.snf.ch > Förderung -> Programme > SCOPES –> Dokumente). Da Sie das Gesuch zusammen mit anderen Personen eingereicht haben, wollen Sie bitte Ihre Informationspflicht gemäss Art. 14 und 32 ff. des "Beitragsreglements" beachten.

Wir bitten Sie, in mySNF den "Antrag auf Beitragsfreigabe" online einzureichen (www.mysnf.ch).

Für die Realisierung Ihres Vorhabens wünschen wir Ihnen viel Erfolg.

Freundliche Grüsse

E. Glättli

Dr. Evelyne Glättli

Aufteilung der Zusprache nach Rubriken

	Total	1. Tranche	2. Tranche	3. Tranche
Schweiz				
Forschungsmittel	60'000	20'000	20'000	20'000
Saläre	0	0	0	0
Sozialabgaben	0	0	0	0
Subtotal	60'000	20'000	20'000	20'000
Partner Ländergruppe A				
Apparate	0	0	0	0
Forschungsmittel	120'000	40'000	40'000	40'000
Subtotal	120'000	40'000	40'000	40'000
Partner Ländergruppe B				
Apparate	0	0	0	0
Forschungsmittel	60'000	20'000	20'000	20'000
Subtotal	60'000	20'000	20'000	20'000
Total	240'000	80'000	80'000	80'000

Beginn: 1. September 2014 Dauer: 36 Monate

Informationen und allgemeine Bedingungen

Der Beitrag ist in Jahrestranchen aufgeteilt. Wir können im Falle von Kürzungen der Bundesmittel, die das Budget des SNF tangieren, Reduktionen bei den Tranchen nicht ganz ausschliessen. Dies gilt nicht für die erste Tranche, die Ihnen im vollen Umfang zugesichert ist.

Die Freigabe der SNF-Beiträge erfolgt unter folgenden zusätzlichen Bedingungen:

- Eine Zusammenfassung der Forschungsarbeiten (lay-summary) ist dem SNF zur Verfügung gestellt worden (vgl. Ziffer 2.2, Reglement über die Information, die Valorisierung und die Rechte an den Forschungsresultaten). Bitte übermitteln Sie die Zusammenfassung online in mySNF unter „lay summary" in Ihrem Gesuch;
- Bei bewilligungs- oder meldepflichtigen Forschungsvorhaben liegen Kopien der gültigen Bewilligungen oder Bestätigungen vor.

Der SNF verpflichtet die von ihm geförderten Forschenden, dass sie eine vollständige Fassung aller publizierten und peer-reviewed Zeitschriftenartikel auf der eigenen Homepage oder dem Hochschulserver hinterlegen, sofern dem keine rechtlichen Bedenken entgegenstehen (vgl. Ziff. 4, Reglement über die Information, die Valorisierung und die Rechte an den Forschungsresultaten) sowie www.snf.ch > Fokus Forschung > Themendossiers > Open access).

Zum Projekt gehören als weitere Beitragsempfängerinnen und Beitragsempfänger:

- Vladislav Volarevic, University of Kragujevac, 34000 Kragujevac
- Ivanka Dimova, Medical University Sofia, Sofia
- Prof. Nenad Filipovic, University of Kragujevac, 34000 Kragujevac

Sicherheit der Projektmitarbeitenden

Der Forschungsrat kann nicht ausschliessen, dass die vom Beitragsempfänger/ von der Beitragsempfängerin geplante Forschung im Partnerland unter dem Aspekt der persönlichen Sicherheit der daran beteiligten Personen ein gewisses Risiko beinhaltet. Er bewilligt das vorliegende Gesuch nur unter dem ausdrücklichen Vorbehalt, dass der Beitragsempfänger/die Beitragsempfängerin für die Sicherheit der beteiligten Forschenden die ihm zumutbaren erforderlichen und zweckmässigen Vorkehrungen trifft, um zu vermeiden, dass Forschende im Rahmen ihrer Tätigkeit für das Forschungsprojekt persönlich oder materiell zu Schaden kommen. Der Nationalfonds lehnt jede Verantwortung oder Haftung für allfällige Schäden des Beitragsempfängers/der Beitragsempfängerin und der Projektmitarbeitenden ab.

Zugang zu externen Gutachten in mySNF

Der Schweizerische Nationalfonds macht die externen Gutachten, welche in der Evaluation eines Gesuchs berücksichtigt wurden, den Gesuchstellenden im Volltext zugänglich. Ausgenommen sind Passagen, welche die Identität des Gutachters bzw. der Gutachterin offenlegen könnten – solche Passagen sind in den Gutachten unkenntlich gemacht.

Sie finden die Gutachten zu Ihrem Gesuch ab dem 28. März 2014 in mySNF unter "Dokumente>Anonymisierte Gutachten".[1]

Die Evaluationsgremien des SNF bemühen sich um eine ausgewogene Gesamteinschätzung jedes Gesuchs. Die externen Gutachten spielen dabei eine zentrale Rolle. Sie beziehen sich allerdings einzig auf die Evaluationskriterien, und die gutachtende Person beurteilt in der Regel nur ein einziges Gesuch. Die Evaluationsgremien des SNF hingegen müssen die Qualität aller zeitgleich eingereichten Gesuche vergleichend beurteilen. Zudem sind die Gutachten häufig generell positiv formuliert, oder sie können einzelne kritische Kommentare enthalten, welche für die Beurteilung der Evaluationsorgane nicht oder wenig relevant waren. Die externen Gutachten bilden deshalb nicht notwendigerweise den Entscheid der Evaluationsgremien des SNF ab.

Der SNF stellt Ihnen die Gutachten als Information zur Verfügung und erwartet keine Antwort Ihrerseits.

[1] Die Nummerierung der Gutachten in mySNF stellt keine inhaltliche Gewichtung dar; sie widerspiegelt lediglich die Reihenfolge, in welcher der SNF die Gutachtenden kontaktiert hatte. Ausgelassene Nummern sind entweder darauf zurückzuführen, dass ein Gutachter bzw. eine Gutachterin ein angekündigtes Gutachten nicht übermittelt hat, oder dass der SNF ein Gutachten aufgrund eines Interessenkonflikts des Gutachters bzw. der Gutachterin nicht hat berücksichtigen können.

Relevante Rechtsdokumente
(www.snf.ch > Porträt > Statuten & Rechtsgrundlagen)

Insbesondere:

- Beitragsreglement
- Allgemeines Ausführungsreglement zum Beitragsreglement
- Reglement über die Information, die Valorisierung und die Rechte an den Forschungsresultaten

Rechtsmittelbelehrung

Gegen diese Verfügung kann gemäss Artikel 13 des Bundesgesetzes vom 14. Dezember 2012 über die Förderung der Forschung und der Innovation (SR 420.1) innerhalb von 30 Tagen nach Eröffnung Beschwerde beim Bundesverwaltungsgericht, Postfach, 9023 St. Gallen, eingereicht werden.

Die Beschwerdeschrift hat die Begehren, deren Begründung mit Angabe der Beweismittel und die Unterschrift der Beschwerdeführerin bzw. des Beschwerdeführers oder der Vertreterin bzw. des Vertreters zu enthalten.

Die angefochtene Verfügung und die als Beweismittel angerufenen Urkunden sind beizulegen, soweit die Beschwerdeführerin bzw. der Beschwerdeführer sie in Händen hat.

www.snf.ch
Wildhainweg 3, Postfach 8232, CH-3001 Bern

Gesuchsformular mySNF

Instrument **Joint research projects (SCOPES)**

1. Teil: Allgemeine Angaben

Eckdaten

Titel des Projekts	ROLE OF BLOOD FLOW AND SDF-1/CXCR4–INDUCED RECRUITMENT OF MONONUCLEAR CELLS IN INTUSSUSCEPTIVE ANGIOGENESIS
Titel in Englisch	ROLE OF BLOOD FLOW AND SDF-1/CXCR4–INDUCED RECRUITMENT OF MONONUCLEAR CELLS IN INTUSSUSCEPTIVE ANGIOGENESIS

Forschungsbereich	Medizin
Hauptdisziplin	30103 Zellbiologie, Zytologie
Hochschule	Universität Bern - BE

Gesuchsteller/innen

Hauptgesuchsteller/in	**Valentin Djonov**
Weitere Gesuchsteller/innen	Vladislav Volarevic
	Ivanka Dimova
	Nenad Filipovic
	Valentin Djonov

Gesuch

Verlangter Betrag (CHF)	Total	**240'000**

Beantragter Beginn	**01.09.2014**
Gewünschte Dauer (Mte)	**36**

Anhänge

Forschungsplan	SciencePart_ValentinDjonov.pdf
Lebenslauf und Publikationsliste	CV_Publist_ValentinDjonov.pdf
	CV_Publist_VladislavVolarevic.pdf
	CV_Publist_NenadFilipovic.pdf
	CV_Publist_IvankaDimova.pdf
Begleitschreiben	CoverLetter_Djonov.pdf

Per Post nachgereichte Dokumente

Research plan
CV and publication list of all partners
Cover letter

1. Hauptgesuchsteller/in in der Schweiz

Name	**Djonov**
Vorname	**Valentin**
Funktion (Titel)	Chair, Institute of Anatomy
Akademischer Grad	Prof.
Geburtsdatum	23.03.1961
Geschlecht	male
Zivilstand	Married
Sprache	German
Nationalität	Switzerland
Korrespondenzanschrift Gesuch	Arbeitsadresse

Privatadresse

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Strasse, Nr.	Mättelistrasse 17
Postfach	
PLZ	3122
Ort	Kehrsatz
Land	Switzerland

Instituts-Anschrift

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2. Weitere Gesuchsteller/innen in der Schweiz und Partnerlandand

Allgemeine Angaben

Name	**Volarevic**
Vorname	**Vladislav**
Funktion (Titel)	
Akademischer Grad	
Geburtsdatum	09.09.1979
Geschlecht	männlich
Zivilstand	
Sprache	Englisch
Nationalität	Serbien und Montenegro
Anschrift(en)	Eigene Arbeitsadresse erfassen

Instituts-Anschrift

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Kommunikation

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Name	**Dimova**
Vorname	**Ivanka**
Funktion (Titel)	
Akademischer Grad	
Geburtsdatum	14.12.1973
Geschlecht	weiblich
Zivilstand	
Sprache	Englisch
Nationalität	Bulgarien
Anschrift(en)	Eigene Arbeitsadresse erfassen

Instituts-Anschrift

Bezeichnung 1 (z.B. Labor) *	Department of Medical genetics
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Land	Bulgarien

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Funktion (Titel)	
Akademischer Grad	
Geburtsdatum	23.02.1970
Geschlecht	männlich
Zivilstand	
Sprache	Englisch
Nationalität	Serbien und Montenegro
Anschrift(en)	Eigene Arbeitsadresse erfassen

Instituts-Anschrift

Bezeichnung 1 (z.B. Labor) *	
Bez. 2 (z.B. Inst. / Dept.)	Faculty of Mechanical Engineering
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Allgemeine Angaben

Name	**Djonov**
Vorname	**Valentin**

Funktion (Titel)	
Akademischer Grad	
Geburtsdatum	23.03.1961
Geschlecht	männlich
Zivilstand	
Sprache	Deutsch
Nationalität	Schweiz
Anschrift(en)	Eigene Arbeitsadresse erfassen

Instituts-Anschrift

Bezeichnung 1 (z.B. Labor) *	Institute of Anatomy
Bez. 2 (z.B. Inst. / Dept.)	
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Land	Schweiz

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E-Mail-Adresse	djonov@ana.unibe.ch

3. Grunddaten I

Original-Titel	ROLE OF BLOOD FLOW AND SDF-1/CXCR4–INDUCED RECRUITMENT OF MONONUCLEAR CELLS IN INTUSSUSCEPTIVE ANGIOGENESIS
Titel in Englisch	
Beantragter Beginn	01.09.2014
Gewünschte Dauer (Mte)	36
Forschungsbereich	Medizin
Haupt-Disziplin	30103 Zellbiologie, Zytologie
Neben-Disziplin(en)	30102 Molekularbiologie
	30303 Herz- und Kreislaufforschung
	30401 Experimentelle Krebsforschung
	30403 Immunologie, Immunpathologie

4. Grunddaten II

Zusammenfassung

Intussusceptive angiogenesis (IA) known also as splitting angiogenesis is a recently described mechanism of vascular growth complementary to sprouting. Intussusceptive angiogenesis is a specific mode of blood vessel formation by which instead of extraluminally, endothelial cells form intraluminal “sprout like” protrusions” resulting in the formation of transluminal endothelial pillars. Successive reshaping and fusion of such pillars “splits” the pre-existing segment in two parts, thus resulting in the formation of an additional vessel segment. IA plays an essential role in vascular remodeling and adaptation of vessels during normal and pathological angiogenesis. It is an “escape” mechanism during and after irradiation and anti-VEGF therapy. Both aforementioned treatments induceangiogenic switch from sprouting to IA by formation of multiple transluminal tissue pillars in the enlarged sinusoidal-like vessels. Our data revealed that IA was significantly induced after inhibition of Notch signaling resulting in an increased capillary density by more than 50%. The induced IA was associated with detachment of pericytes from basement membranes, increased vessel leakage and recruitment of mononuclear cells to the sites of pillars; the process was dramatically enhanced when we inject bone marrow-derived mononuclear cells. The leading event in triggering of IA was the extravasation of mononuclear cells of bone marrow origin, which was associated with up-regulation of chemotaxis factors SDF-1 and CXCR4. In addition, SDF-1 expression was up-regulated in the endothelium of liver sinusoids in Notch1 knockout mice mouse, together with vascular remodeling by intussusception. Our hypothesis is that SDF-1/CXCR4 signaling is crucial factor in the process of intussusceptive angiogenesis. The last is promoted by shear stress as well, since it occurs only after the establishment of blood flow. There is evidence that SDF-1–positive dermal vascular endothelium can promote specific recruitment of CXCR-4–positive cells under conditions of physiologic shear flow.
We would like to obtain novel insights into the regulation mechanisms of intussusceptive angiogenesis and translate the knowledge obtained to clinical relevant topics such as as tissue regeneration and tumor growth.
The aim of our project is to study the role of blood flow and SDF-1/CXCR4–induced recruitment of mononuclear cells in intussusceptive angiogenesis using both experimental models and numerical simulations.
During the next 3 years we aim to focus on the following fundamental aspects: (i) the role of SDF-1/CXCR4 signaling in intussusceptive angiogenesis, (ii) the role of the bone marrow derived/peripheral blood mononuclear cells in IA during tissue regeneration and their contribution to the “escape” mechanism after anti VEGF therapy and irradiation in cancer, iii) SDF-1/CXCR4 interactions with blood flow/shear stress in the process of intussusception.
In accomplishing our objectives we will use different models and approaches: (i) in vivo pharmacological studies on chicken area vasculosa, (ii) in vivo genetic studies in zebrafish embryo, (iii) mouse model of liver intussusceptive microvascular growth, (iv) mouse tumor xenografts, switching to intussusceptive angiogenesis after irradiation and anti-angiogenic treatment, (v) detailed morphological and computer modeling, molecular, immunological and cellular analyses for different types of cells (macrophages, neutrophils, eosinophils, mast cells, dendritic cells, NK cells, T- and B-cells).

	We anticipate that this project will yield important information regarding intussusceptive angiogenesis and role of bone marrow derived/peripheral blood cells. Our data will help us to design more effective pro- and anti-angiogenic treatment strategies.
Keywords	intussusceptive angiogenesis
	SDF-1 /CXCR4 signaling
	bone marrow derived cells in angiogenesis
Korrespondenzsprache	Deutsch
Beitragsverwaltende Stelle	Universität Bern Finanzabteilung

5. Direkte Beziehungen zu anderen Gesuchen

6. Hochschule

Hochschule	Universität Bern - BE
Bemerkungen	

7. Zusammenarbeit (national und international)

8. Vorhandene oder beantragte Mittel

Stehen Ihnen weitere Mittel für das Projekt zur Verfügung?	Ja
Personal oder Stellen	One technician Institute of Anatomy Bern One PhD Student Sofia One technician Serbia
Vorhandene Apparate	Entire Equipment of the 3 Partners
Beiträge für neue Apparate, Verbrauch, Reisen und weitere Ausgaben	no
Infrastruktur	Entire Infrastructure of the 3 Partners

Haben Sie im Kontext des Projekts beim SNF (verantwortliche/r oder weitere/r GesuchstellerIn) oder bei anderen Geldgebern weitere Mittel beantragt?	Nein

9. Beantragte Stellen, Saläre, Sozialabgaben

Übersicht Stellen (Vollzeitäquivalente)

Rubrik	Total %	Jahr 1	Jahr 2	Jahr 3
Akademiker/in				
Doktorand/in				
Techniker/in				
Hilfskraft				
Total %	**0**	**0**	**0**	**0**

Details

Nachname	Nationalität	Akademischer Grad	Funktion	Lohnklasse	Salär anderer, 1. Jahr	Salär SNF, 1. Jahr
Vorname	**Geschlecht**	**Seit**	**Stellenprozent**	**Seit**	**2. Jahr**	**2. Jahr**
Geburtstag	**Anzahl Kinder**	**Doktorand seit**	**Bereits vom SNF bezahlt**	**Stellenkategorie**	**3. Jahr**	**3. Jahr**
Subprojekt					**4. Jahr**	**4. Jahr**
					5. Jahr	**5. Jahr**
					6. Jahr	**6. Jahr**

Stellentotals CHF

Rubrik	
Akademiker/in	
Doktorand/in	
Techniker/in	
Hilfskraft	
Total CHF	

10. Finanzieller Bedarf

Budget pro Jahr (Übersicht)

Schweiz

Rubrik	Total SNF (CHF)	Jahr 1	Jahr 2	Jahr 3
Forschungsmittel	60'000	20'000	20'000	20'000
Saläre				
Sozialabgaben				
Total SNF (CHF)	**60'000**	**20'000**	**20'000**	**20'000**

Budget pro Jahr (Übersicht)

Partner Ländergruppe A

Rubrik	Total SNF (CHF)	Jahr 1	Jahr 2	Jahr 3
Apparate				
Forschungsmittel	120'000	40'000	40'000	40'000
Total SNF (CHF)	**120'000**	**40'000**	**40'000**	**40'000**

Budget pro Jahr (Übersicht)

Partner Ländergruppe B

Rubrik	Total SNF (CHF)	Jahr 1	Jahr 2	Jahr 3
Apparate				
Forschungsmittel	60'000	20'000	20'000	20'000
Total SNF (CHF)	**60'000**	**20'000**	**20'000**	**20'000**
Total SNF (CHF)	**240'000**	**80'000**	**80'000**	**80'000**

Budget pro Jahr (Detail)

Nicht beim SNF beantragte Mittel (in Klammern) werden nicht zum Total addiert.

Keine Subprojekte

	Total SNF (CHF)	Jahr 1	Jahr 2	Jahr 3
Schweiz				
Forschungsmittel				
Consumables: 19000 (1st year); 18000 (2nd year); 17000 (3rd year) Travel: 1000 (1st year); 1000 (2nd year); 1000 (3rd year) Publication: 1000 (2nd year); 2000 (3rd year)	60'000	20'000	20'000	20'000
Partner Ländergruppe A				
Forschungsmittel				
Consumables: 38000 (1st year); 36000 (2nd year); 36000 (3rd year) Travel: 2000 (1st year); 4000 (2nd year); 4000 (3rd year)	120'000	40'000	40'000	40'000
Partner Ländergruppe B				
Forschungsmittel				
Consumables: 18000 (1st year); 18000 (2nd year); 17000 (3rd year) Travel: 2000 (1st year); 2000 (2nd year); 2000 (3rd year) Publication: 1000 (3rd year)	60'000	20'000	20'000	20'000
Total SNF (CHF)	**240'000**	**80'000**	**80'000**	**80'000**

11. Bewilligungs- oder meldepflichtige Versuche

Forschung mit Menschen	Nein
Forschung mit humanen embryonalen Stammzellen	Nein
Tierversuche	Ja
an Primaten	Nein
an Labornagetieren	Ja
Tierart	mouse
Max. erwarteter Schweregrad	1
Tierversuchsbewilligung	Wird noch eingereicht
an Kaninchen	Nein
an Hunden oder Katzen	Nein
an anderen Säugetieren	Nein
an Nicht-Säugetieren	Nein
Forschung mit GVO oder Pathogenen	Nein
Relevante Bestimmungen zur Kenntnis genommen und akzeptiert	Ja

12. Allgemeine Mitteilungen zum Gesuch

Kurzbezeichnung	
Mitteilung	
Vertraulich	Nein

Proposal full title: **ROLE OF BLOOD FLOW AND SDF-1/CXCR4–INDUCED RECRUITMENT OF MONONUCLEAR CELLS IN INTUSSUSCEPTIVE ANGIOGENESIS**

Proposal acronym: CELLS IN ANG

Type of funding scheme: SCOPES – Joint Research Project

Name of the coordinating person: Prof. Valentin Djonov

List of participants:

Partner no.	Participant name	Country
1. Co-ordinator	Prof. Valentin Djonov Institute of Anatomy, University of Bern	Switzerland
2. Country A	Prof. Vladislav Volarevic Centre for Molecular Medicine and Stem Cell Research, University of Kragujevac	Serbia
3. Country A	Prof. Nenad Filipovic Center for Bioengineering Faculty of Engineering, University of Kragujevac	Serbia
4. Country B	Assoc. Prof. Ivanka Dimova Department of Medical Genetics, Medical University of Sofia	Bulgaria

Table of content

1. SUMMARY

Intussusceptive angiogenesis (IA) known also as splitting angiogenesis is a recently described mechanism of vascular growth complementary to sprouting. Intussusceptive angiogenesis is a specific mode of blood vessel formation by which instead of extraluminally, endothelial cells form intraluminal “sprout like” protrusions” resulting in the formation of transluminal endothelial pillars. Successive reshaping and fusion of such pillars “splits” the pre-existing segment in two parts, thus resulting in the formation of an additional vessel segment. IA plays an essential role in vascular remodeling and adaptation of vessels during normal and pathological angiogenesis. It is an “escape” mechanism during and after irradiation and anti-VEGF therapy. Both aforementioned treatments induceangiogenic switch from sprouting to IA by formation of multiple transluminal tissue pillars in the enlarged sinusoidal-like vessels. Our data revealed that IA was significantly induced after inhibition of Notch signaling resulting in an increased capillary density by more than 50%. The induced IA was associated with detachment of pericytes from basement membranes, increased vessel leakage and recruitment of mononuclear cells to the sites of pillars; the process was dramatically enhanced when we inject bone marrow-derived mononuclear cells. The leading event in triggering of IA was the extravasation of mononuclear cells of bone marrow origin, which was associated with up-regulation of chemotaxis factors SDF-1 and CXCR4. In addition, SDF-1 expression was up-regulated in the endothelium of liver sinusoids in Notch1 knockout mice mouse, together with vascular remodeling by intussusception. Our hypothesis is that SDF-1/CXCR4 signaling is crucial factor in the process of intussusceptive angiogenesis. The last is promoted by shear stress as well, since it occurs only after the establishment of blood flow. There is evidence that SDF-1–positive dermal vascular endothelium can promote specific recruitment of CXCR-4–positive cells under conditions of physiologic shear flow.

We would like to obtain novel insights into the regulation mechanisms of intussusceptive angiogenesis and translate the knowledge obtained to clinical relevant topics such as as tissue regeneration and tumor growth.

The aim of our project is to study the role of blood flow and SDF-1/CXCR4–induced recruitment of mononuclear cells in intussusceptive angiogenesis using both experimental models and numerical simulations.

During the next 3 years we aim to focus on the following fundamental aspects: (i) the role of SDF-1/CXCR4 signaling in intussusceptive angiogenesis, (ii) the role of the bone marrow derived/peripheral blood mononuclear cells in IA during tissue regeneration and their contribution to the “escape” mechanism after anti VEGF therapy and irradiation in cancer, iii) SDF-1/CXCR4 interactions with blood flow/shear stress in the process of intussusception.

In accomplishing our objectives we will use different models and approaches: (i) in vivo pharmacological studies on chicken area vasculosa, (ii) in vivo genetic studies in zebrafish embryo, (iii) mouse model of liver intussusceptive microvascular growth, (iv) mouse tumor xenografts, switching to intussusceptive angiogenesis after irradiation and anti-angiogenic treatment, (v) detailed morphological and computer modeling, molecular, immunological and cellular analyses for different types of cells (macrophages, neutrophils, eosinophils, mast cells, dendritic cells, NK cells, T- and B- cells).

We anticipate that this project will yield important information regarding intussusceptive angiogenesis and role of bone marrow derived/peripheral blood cells. Our data will help us to design more effective pro- and anti-angiogenic treatment strategies.

2. RESEARCH PLAN (Max. 15 pages)

2.0. INTRODUCTION

Angiogenesis is essential for normal embryonic development and reproductive cycle, and plays a key role in pathological conditions such as tumor growth and ischemic cardiovascular diseases. This is a complex process involving essential signaling pathways, for instance VEGF, bFGF and Notch etc. in vasculature, as well as additional players such as bone marrow-derived endothelial progenitor cells. The better understanding for the role of the different pathways and the crosstalk between different cells during angiogenesis is a crucial factor for developing of more effective pro-angiogenic and anti-angiogenic anticancer therapy.

Angiogenesis involves the formation of new blood vessels from a pre-existing vascular plexus, and based on morphological characteristics two main distinct processes have been identified: sprouting and intussusceptive angiogenesis (IA) (Ribatti D & Crivellato E, 2012; **Djonov V** et al, 2000; **Djonov V** et al, 2003). Sprouting angiogenesis has been well described since more than 150 years ago. Recent publications indicated that sprouting involves tip/stalk cell differentiation and crosstalk processes, which are tightly controlled by the VEGF and Notch/Dll4 signalling pathway (Jakobsson L et al, 2010; Blanco R & Gerhardt H, 2013). Intussusceptive angiogenesis is a specific mode of blood vessel formation by which, endothelial cells form intraluminal “sprout like protrusions” resulting in the formation of transluminal endothelial pillars. Successive reshaping and fusion of such pillars “splits” the pre-existing segment in two parts, thus resulting in the formation of an additional vessel segment. This results in formation of supplying and draining vessels, pruning of arteries and veins, and finally remodeling of the primitive capillary plexuses. IA is a step-process involving intussusceptive microvascular growth, intussusceptive arborization and intussusceptive remodeling (**Djonov V** et al, 2000; **Djonov V** et al, 2003; **Makanya AN** et al, 2009; **Styp-Rekowska B** et al, 2011). The processes lead to formation of hierarchically organized and mature vascular networks. Compared to sprouting angiogenesis, intussusception is relatively fast, thus allowing swift vascular adaptation in compromised conditions. In liver regeneration models and in post-pneumonectomy alveolar angiogenesis, intussusception has been identified as the predominant mode of growth, and also in extra-embryonic vasculatures including the vitelline circulation, intussusception appears to prevail above sprouting (Dill MT et al, 2012; **Baum O** et al, 2010; Konerding MA et al, 2012). The intensive work of our group in the last few decades clearly documented the morphological features of this specific angiogenic mode and demonstrated its definite presence during development and tumorigenesis as a complementary to sprouting vessel growth. Surprisingly, **the cellular and molecular regulation of intussusception is less well known** but recent evidence suggests that it might involve a component regulated by blood flow and Notch signaling (**Styp-Rekowska B** et al, 2011; Dill MT et al, 2012). We provided evidence showing that Notch regulates intussusception involving interaction with circulating mononuclear cells in developing vascular networks (**Dimova I** et al, 2013). We now directed our research to unravel the cellular and molecular characteristics of intussusceptive angiogenesis.

Our preliminary results suggest that intussuception is most probably synchronized by chemokine factors since intussusceptive growth was associated with the recruitment of mononuclear cells (**Dimova I** et al, 2013). After injection of bone marrow derived mononuclear cells we observed robust induction of intussusception in Notch inhibited samples. Notably, the chemotactic factors SDF-1/CXCR4 were up-regulated specifically due to Notch inhibition. Our hypothesis is that Notch inhibition disturbed vessel stability and led to pericyte detachment followed by extravasation of mononuclear cells due to the activation of the SDF-1/CXCR4 axis. The stromal cell-derived factor SDF-1 binds to its receptor CXCR4 and directs migration of progenitor cells into the appropriate sites. The mononuclear cells contribute to formation of transluminal pillars with sustained IA resulting in a dense vascular plexus. Recent studies have demonstrated that bone marrow-derived cells home at the sites of neovascularization in response to tissue-derived or circulating cytokines and their precise angiogenic role is still elusive.

In a number of in vivo studies, high-level expression of SDF-1 in a target tissue is critical to the generation of a chemokine gradient across the endothelium, which then promotes transendothelial migration of SDF-1 receptor-expressing cells. Once present on the surface of endothelial cells, SDF-1 could support lymphocyte arrest onto the vascular endothelium. Yao L et al provided evidence that SDF-1–positive dermal vascular endothelium can promote specific arrest of CXCR-4–positive cells

under conditions of physiologic shear flow (Yao L et al, 2003). In adition, shear stress up-regulated the secretion of stromal-derived factor-1 (Yuan L et al, 2013).

Changes in hemodynamic forces, especially wall shear stress, have been shown to influence the process of angiogenesis (Bongrazio et al. 2000; Garcia-Cardena et al. 2001, Lammerding and Lee 2009; Buschmann et al. 2010; Eichmann et al. 2004). One of the roles of shear stress is to ensure the integrity of the walls of vessels, but it is also known that shear stress plays a role in several processes that are involved in intussusceptive angiogenesis; for example shear stress can inhibit the formation of tubules, the migration of endothelial cells and also increase the bundles of endothelial actin filaments. During the process of angiogenesis, the wall shear stress in the observed domain changes significantly and it was shown that the increase of wall shear stress can initiate the process of intususceptive angiogenesis in skeletal muscles (Baum et al. 2004; Milkiewicz et al. 2005). However, the exact connection between shear stress changes and the regulation of angiogenesis are still unknown. The precise mechanism by which a physical force is converted into a chemical signal in these cells is not clear. It is well documented that autologously secreted chemokines can form local pericellular diffusion gradients scattered by fluid convection, and cells subsequently chemotaxis up the flow-directed gradient (Shields JD et al, 2007; Randolph GJ et al, 2005). Shear stress promoted human mesenchymal stem cell (hMSC) migration through an up-regulated SDF-1/CXCR4 axis, whereas after covering the wound area, the cell migration ability was reduced and further produced a ‘‘feedback signal’’ to reduce SDF-1 productions as well as CXCR4 expressions (Yuan L et al, 2013).

The aim of our project is to study the role of blood flow and SDF-1/CXCR4–induced recruitment of mononuclear cells in intussusceptive angiogenesis using both experimental models and numerical simulations.

2.1. CURRENT STATE OF RESEARCH IN THE FIELD

2.1.1. Previous work on the role of bone marrow-derived/peripheral blood cells in angiogenesis

We have largely expanded our knowledge about the role of bone marrow-derived (BMD) cells in stimulating angiogenesis after their discovery in 1997 (Asahara et al., 1997) and now their capability to promote vessel formation is intensively investigated. The domain comes to be multifaceted and contradictory data were sometimes arising.

First it was proposed and evidence was provided that myeloid cells can turn into endothelial cells in hypoxic tissue demand. Asahara et al (1997) reported that purified CD34+ hematopoietic progenitor cells in adults can differentiate ex vivo to an endothelial phenotype. The cells were at the same time positive for VEGFR2, a specific endothelial marker and they were named endothelial progenitor cells (EPC). Thus EPC express both hematopoietic stem cell and endothelial cell markers on their surface (Zhao YH et al, 2013). The intensive studies in the last years allowed distinguishing subpopulations of mononuclear cells existing in the adult bone marrow and circulating in peripheral blood, which support angiogenesis without incorporating permanently into the newly formed vessel - circulating angiogenic cells (CAC) (Fang S, Salven P, 2011). Currently, bone marrow derived (BMD) cellular populations with angiogenic properties are classified according to their phenotypic markers in the following groups: (i) EPC, which express VEGFR2, Tie2, CXCR4, CD31, CD34, CD133 (for immature progenitor cells) and they are negative for CD14; (ii) Monocytes, which express CD14 and have different subclasses such as positive for Tie2, CXCR4, VEGFR2 or VEGFR1; and (iii) Macrophages, mostly positive for CXCR4 and VEGFR1 (Favre J et al, 2013).

The discovery that mononuclear cells can home to sites of hypoxia and enhance neo-angiogenesis has faced the possibility of using the isolated hematopoietic stem cells or EPC for therapeutic vasculogenesis (Wara AK et al, 2011). Remarkably, infusion of terminally differentiated mature endothelial cells did not improve neovascularization (Kocher AA et al, Nat Med. 2001; Hur J et al, Arterioscler Thromb Vasc Biol. 2004) suggesting that a not-yet-defined functional characteristic (eg, chemokine or integrin receptors mediating homing) is essential for EPC-mediated vascular augmentation after ischemia (Cochain C et al, 2010). During endothelial repair after vascular injury and during tumor angiogenesis, BMDC do not seem to be involved in re-endothelialization stressing rather their supportive role over trans-differentiation (Dudley AC et al, 2010; Hagensen MK et al, 2012).

Monocytic cells may play a crucial role also in collateral growth by adherence to the vascular wall during both arteriogenesis and tumour angiogenesis (Murdoch C et al, 2008). These data suggest that

monocytic cells are necessary for arteriogenesis and possibly neovascularization. For therapeutic application, the local enhancement of monocyte recruitment might be better suited than systemic infusion of monocytic cells, which only leads to a relatively minor increase in the number of circulating monocytes.

Although the role of BMD and peripheral blood mononuclear (PBM) cells in neovascularization has been convincingly shown by several groups, the question remains: how do these cells improve neovascularization? The efficiency of neovascularization may not solely be attributable to the incorporation of these cells in newly formed vessels, but may also be influenced by the release of pro-angiogenic factors in a paracrine manner (Ribatti D, 2009). It was recently shown that secreting factors from peripheral blood mononuclear cells enhance neo-angiogenesis (Mildner M et al, 2013). The capacity of EPC to physically contribute to vessel-like structures may contribute to their potent capacity to improve neovascularization as well (Urbich C et al, Circulation 2003). Further studies are to elucidate the contribution of physical incorporation, paracrine effects and possible effects on vessel remodeling and facilitating vessel branching to EPC-mediated improvement of neovascularization are required. Likely, paracrine effects contribute in addition, to the physical incorporation of EPC into newly formed capillaries. The influence of the incorporation of a rather small number of circulating cells on remodeling and vessel maturation has to be further elucidated.

Another open question is what drives BMD and PBM cells recruitment to the sites of angiogenesis? Ischemia is believed to up-regulate VEGF or SDF-1 (Lee SH et al, N Engl J Med. 2000), which in turn are released to the circulation and induce mobilization of progenitor cells from the bone marrow via a MMP-9– dependent mechanism (Heissig B et al, Cell. 2002; Shintani S et al, Circulation. 2001). Indeed, SDF-1 has been proven to stimulate recruitment of progenitor cells to the ischemic tissue (Yamaguchi J et al, Circulation. 2003). SDF-1 protein levels were increased during the first few days after induction of myocardial infarction (Askari AT et al, Lancet. 2003). Moreover, overexpression of SDF-1 augmented stem cell homing and incorporation into ischemic tissues (Yamaguchi J et al, Circulation. 2003; Askari AT et al, Lancet. 2003). Interestingly, hematopoietic stem cells were shown to be exquisitely sensitive to SDF-1 and did not react to G-CSF or other chemokines (eg, IL-8 and RANTES) (Wright DE et al, J Exp Med. 2002). The migratory capacity of EPC or bone marrow cells toward VEGF and SDF-1, respectively, determined the functional improvement of patients after stem cell therapy (Britten MB et al, Circulation.2003).

SDF-1/ CXCR4 axis is crucial in the homing mechanisms of hematopoietic cells and the metastasis of solid tumors. In the past few years, numerous studies have focused on studying the role of this signaling in angiogenesis and proved its angiogenic activity in organ repair and tumor development. However, the precise mechanisms by which SDF-1 exerts its pro-angiogenic effects are not fully elucidated. Since it is supposed to be an angiogenic growth factor, it is a good candidate for pro- and anti-angiogenic therapy. It was reported that transient disruption of the SDF-1/CXCR4 axis using CXCR4 blocking antibody blocked the recruitment of bone marrow-derived cells into the angiogenic sites of tumor tissue, and resulted in complete inhibition of accelerated tumor growth after chemotherapy in mouse (Murakami J, et al, 2009). SDF-1 is constitutively expressed in the bone marrow and various tissues, which enables it to regulate the trafficking, localization and function of immature and mature leukocytes, including monocytes, neutrophils, dendritic cells and T lymphocytes (Karin N et al, 2010). All these immune cells play important role in tumor angiogenesis and vascularization. However, the precise role of SDF-1/CXCR4 axis on function of monocytes/macrophages, neutrophils, DC, T lymphocytes in the process of intussusceptive angiogenesis is completely unknown and will be evaluated in this study.

Several clinical studies have shown a correlation between a high number of tumor-associated **macrophages** and increased microvessel density, suggesting that these cells might promote tumor angiogenesis, particularly due to production of pro-angiogenic and angiogenesis modulating factors (reviewed in Murdoch C et al, 2008). A number of functional in vitro and in vivo studies demonstrate that tumors stimulate **neutrophils** to promote angiogenesis and immunosuppression, as well as migration, invasion and metastasis of the tumor cells (Dumitru CA et al, 2013). In inflammation, the SDF-1/CXCR4 signaling pathway plays an important role in the modulation of neutrophil activity, not only by promoting chemotaxis but also by suppressing cell death (Yamada M et al, 2011). Although limited, there is evidence to suggest that tumor-infiltrating **eosinophils** can influence angiogenesis (reviewed in Murdoch C et al, 2008). Freshly isolated human blood eosinophils or supernatants from

cultured eosinophils induce endothelial cell proliferation in vitro and angiogenesis in the rat aortic ring assay, suggesting that eosinophils can directly influence angiogenesis. The high number of **mast cells** (MC) has been observed in various tumors where increased MC density positively correlates with increased microvessel density (reviewed in Murdoch C et al, 2008). **Dendritic cells** (DC) promote tumor angiogenesis both by their secretion of pro-angiogenic cytokines (VEGF, IL-8, TNF-alpha) and their ability to serve as a local supply of endothelial progenitors (Strioga M et al, 2013). **Natural killer** (**NK) cells** control both local tumor growth and metastasis and participate in cancer elimination by inhibiting cellular proliferation and angiogenesis (Levy EM et al, 2011). **T helper (Th) cells** -mediated immunity has traditionally been viewed as favoring tumour growth, both by promoting angiogenesis and by inhibiting cell-mediated immunity and subsequent tumour cell killing, there are also many studies demonstrating the anti-tumor activity of CD4+ Th2 cells, particularly in their collaboration with tumor-infiltrating eosinophils or due to direct anti-angiogenic effects of IL-4 (reviewed in Ellyard JI et al, 2007). T regulatory cells (Tregs) are potent immunosuppressive cells that promote progression of cancer through their ability to limit anti-tumor immunity and promote angiogenesis. The accumulation of Tregs in tumors correlates with biomarkers of accelerated angiogenesis such as VEGF overexpression and increased microvessel density, providing clinical cues for an association between Tregs and angiogenesis (reviewed in Facciabene A et al, 2012).

2.1.2. Previous work on intussusceptive angiogenesis

Intussusceptive angiogenesis is well documented and widely spread mode of angiogenesis, occurring both during normal development and in pathological conditions. In contrast to sprouting angiogenesis, whereby abluminal sprouts grow outwards and subsequently merge with the existing capillaries, intussusceptive angiogenesis is elaborated by intraluminal growth of endothelial cell processes. The latter protrude from the opposing sides of the vessel wall and form transluminal tissue pillar, representing endothelial bilayer, which is afterwards perforated and stabilized from outside by collagen bundles. Repetitive formation of pillars and their subsequent fusion lead to the splitting of vessels and vascular expansion. Increase in the girth of pillars forms meshes, thus splitting the vessel. Intussusceptive angiogenesis is a process linked to both blood vessel replication and remodeling **in development**. It is present within the regions of increased vascular density in alveolar angiogenesis during compensatory growth after pneumonectomy in a murine model of post-pneumonectomy lung growth (Konerding MA et al, 2012). The remodeling of the retiform meshworks in the avian lung was essentially accomplished by intussusceptive angiogenesis as well (**Makanya AN** et al, 2011).

In addition to its developmental role, intussusceptive angiogenesis is well documented as a **mechanism of vascular adaptation** in response to different environmental stimuli. In the adult mouse retina it was reported as a main adaptive mechanism **to chronic systemic hypoxia** (Taylor AC et al, 2010). These investigations contribute to our understanding of hypoxia-induced angiogenesis and microvascular remodeling. The process of intraluminal division participates in the inflammation-induced neovascularization associated with chemically induced murine colitis (Konerding MA et al, 2010). SEM of vascular corrosion casts demonstrated replication of the mucosal plexus without significant evidence of sprouting angiogenesis, whereas pillar formation and septation were present within days of the onset of inflammation. The authors conclude that intussusceptive angiogenesis is a fundamental mechanism of microvascular adaptation **to prolonged inflammation**. It is also a **mechanism of compensation** for vascular growth. In capillary regression model of inflamed murine corneas the abrupt termination of capillary sprouting is followed by an intussusceptive response (Peebo BB et al, 2011). The **capillary repair during kidney recovery** in Thy1.1 nephritis was done by intussusceptive angiogenesis (**Wnuk M** et al, 2012). Inhibitors of angiogenesis and radiation induce compensatory changes in the tumor vasculature both during and after cessation of treatment. There is switch from sprouting to intussusceptive angiogenesis, which may be an adaptive response of tumor vasculature **to cancer therapy** that allows the vasculature to maintain its functional properties (**Hlushchuk R** et al, 2008; **Hlushchuk R** et al, 2011; Abdullah SE et al, 2012). Potential candidates for molecular targeting of this angioadaptive mechanism are yet to be elucidated in order to improve the currently poor efficacy of contemporary anti-angiogenic therapies. Important is the involvement of intussusceptive angiogenesis **in pathological conditions**. Vascular remodeling of the hepatic sinusoidal microvasculature **in the course of liver nodular hyperplasia** is a result of intussusceptive growth (Dill MT et al, 2012). This angiogenic mode is widely involved in tumor development. By using electron and confocal microscopy, Paku et al observed intraluminal nascent pillars that contain a collagen bundle covered by endothelial

cells (ECs) **in the vasculature of experimental tumors** (Paku S et al, 2011). Tumor angiogenesis in liver metastasis from colon carcinoma is a controversial subject. Ceauşu RA et al conclude that **in liver metastasis** principal mechanism of neovascularisation formation is based on intussusception (Ceauşu RA et al, 2011). **In metastatic tumors of the brain** there was intussusceptive angiogenesis, whereby the fibrosarcoma cells were attached to the vessel, filled the developing pillars and caused lumen splitting (Bugyik E et al, 2011). Branching angiogenesis was not observed either in the tumors or in control cerebral wounds. These data suggest that sprouting angiogenesis is not needed for the incipient growth of cerebral metastases and that tumor growth in this model is a result of incorporation of host vessels. Prolactin was found to directly stimulate angiogenesis **in breast cancer progression**, enhancing vessel density and the tortuosity of the vasculature by pillar formation, which are hallmarks of intussusceptive angiogenesis (Reuwer AQ et al, 2012). It is a preferred mode of angiogenesis **in oral squamous cell carcinoma** (Oliveira de Oliveira LB et al, 2013) and **in hepatocellular carcinoma** (Géraud C et al, 2013; Piguet AC et al, 2011).

Despite this variety of intussusceptive angiogenic roles, most of the current research is focused on the mechanism of sprouting angiogenesis because this mechanism was first described and because most existing experimental models are related to sprouting angiogenesis. Consequently, the mechanism of intussusceptive angiogenesis is often overlooked in angiogenesis research (De Spiegelaere W et al, 2012). Intussusception is an alternative to the sprouting mode of angiogenesis. The advantage of this mechanism of vascular growth is that blood vessels are generated more rapidly and the capillaries thereby formed are less leaky (Ribatti D et al, 2012). The regulation of intussusceptive angiogenesis is still to be elucidated. There are some hypotheses about the possible drivers of intussusception. In the sprouting type of angiogenesis related to hypoxia, there is no blood flow in the rising capillary sprout. In contrast, it has been shown that an increase of wall shear stress initiates the splitting type of angiogenesis in skeletal muscle (**Styp-Rekowska B** et al, 2011). Inflammation-associated intussusceptive angiogenesis in adult mice was associated with vessel angle remodelling and the morphometry of the vessel angles suggests the influence of blood flow on the location and orientation of remodeled vessels (Ackermann M et al, 2013). Regarding molecular regulation, very little is known for the molecular factors with potential significance. Application of the essential angiogenic factors VEGF and bFGF in arteriovenous loop model demonstrated advanced neovascularisation in the phase of remodelling by a higher incidence of intussusception, compared to control without these factors (Polykandriotis E et al, 2011). It was shown in Ewing sarcomas and rhabdomyosarcomas that treatment suppressing IGF-1 signaling decreases intussusceptive angiogenesis (Ackermann M et al, 2012).

2.1.3 Previos work on wall shear stress effect on intussusceptive angiogenesis

Papers in literature suggest several biological components in cells that could sense the changes in mechanical quantities, such as glykokalyx in endothelial cells (Tarbell and Pahakis 2006; Barakat 2008), force-induced unfolding of extracellular matrix proteins (Smith et al. 2007), the nucleus (Jaalouk and Lammerding 2009) etc. If endothelial cells are exposed to appropriate shear stress, it will induce the VEGFR-2 and stimulate the formation of appropriate receptors and gene transcriptions and activate pathways to stimulate the formation of new blood vessels (Dejana et al. 2000). Zhang et al. 2009 suggested that tetraspanins, a protein family, has an important role in the process of angioadaptation. These proteins are membrane-spanning proteins that attach to other membrane-bound molecules such as cell-adhesion proteins, growth factor receptors, integrins etc. and this way they control important cellular processes (adhesion, migration, fusion). Per example, tetraspanins in connection with integrins can regulate and strengthen the process of adhesion when shear stress changes. On the macroscopic level, tetraspanins ensure that the development and functioning of the vascular system is normalized (Hemler 2008). Several authors suggested there is another important factor in the process of angiogenesis, primary cilium (Groenendijk BC et al, 2004; Van der Heiden K et al, 2006; Nauli SM et al, 2008; Zhou, Q.et al, 2008). Cilium can detect the shear stress changes. Namely, it was detected that endothelial cells possess cilia, but that it disappears if cells are exposed to high values of shear stress (Iomini et al. 2004). Distribution of a high shear stress marker named Krüppel-like-factor-2 (KLF-2) and distribution of primary cilia and mutually inverse, i.e. primary cilia is detected in regions where KLF-2 is not expressed. This means that primary cilia appear in regions where shear stress is expected to be low (Van der Heiden et al. 19-28).

Yuan L et al found that shear stress up-regulated the secretion of stromal-derived factor-1 (SDF-1), which stimulated its receptor CXCR4 expression in hMSCs until the cells covered the wounded area (Yuan L et al, Stem Cells and Development 2013). Treatment with CXCL12 induced expression of both iNOS mRNA and protein in primary human CD8 T cells in a dose-dependent manner. Induction of iNOS expression in CD8 T cells was mediated by increased gene transcription. iNOS expression in infiltrating human CD8 T cells was spatially associated with CXCL12 expression both in the humanized mouse model of allograft artery rejection and in clinical specimens of coronary arteries displaying allograft vasculopathy (Choy J et al, J Heart Lung Transplant 2008). eNOS is activated by SDF-1α and is required for endothelial cell migration induced by SDF-1α (Pi X et al, PNAS 2009). Using BAECs the authors demonstrated that eNOS was phosphorylated by SDF-1 in a time- and dose-dependent manner. Taken together, these data suggest that eNOS moderates SDF-1- triggered cell migration. Shear stress induces SDF-1 expression as well. From another hand SDF-1 also induces eNOS expression – a marker of shear stress.

These are some of the observed factors that either have certain influence on shear stress changes or that can detect these changes. But, additional analysis from the experimental point of view is necessary. Another tool that could be very helpful in gaining more knowledge about mechanical influence of blood flow on the process of angiogenesis are the numerical simulation techniques, such as the finite element method that can be used as a powerful tool to simulate blood flow and determine the distribution of shear stress, fluid velocity and pressure. The essential structural features of vessels are extracted from experimental data and the geometry for the computational model is created. The computational model is able to closely approximate the distribution of physical quantities, with the main goal to provide valuable information about the intravascular flow that could help regulate the process of intussusceptive angiogenesis (**Filipovic** et al 2009).

2.2. PAST PERFORMANCE IN THE RESEARCH FIELDS

The group of Valentin Djonov contributed immensely to the investigation of the angiogenic mechanisms during development and organ recovery after injury, as well as during tumor progression and spread. It is one of the first in the world making careful documentation of the features of intussusceptive angiogenesis. Since more than 15 years they have studied the steps and drivers of intussusception and have more than 150 publications in peer-reviewed journals with more than 4000 citations in high impact factor journals. A full list of publication can be obtained at http://www.ncbi.nlm.nih.gov/pubmed?term=djonov%20v. Recently **Valentin Djonov** together with **Ivanka Dimova** (**Dimova I** et al, 2013) have published results indicating that Notch inhibition disturbs vessel stability and induces intussusceptive neo-angiogenesis, triggering in this way the augmentation of the capillary plexus but without the accompanying vascular maturation and remodeling (Figure 1). It was associated with extravasation of mononuclear cells of bone marrow origin possibly by up-regulation SDF-1/CXCR4 chemotactic factors (Figure 2 and 3). The endothelial expression of SDF-1 was detected in liver of Notch1 knockout mouse as well, whereby it was again associated with intussusception (Figure 4).

The group of Valentin Djonov has already studied the influence of blood flow and hemodynamics forces on the process of intussusceptive angiogenesis and they performed experiments associated with vascular growth both *in vitro* and *in vivo* (**Burri** et al, 2004; **Djonov** et al, 2002; **Makanya** A et al, 2009; **Djonov V** et al, 2003). The role of blood flow in regulation of the process of IA was demonstrated in several experiments. One of them included occlusion of one of the dichotomous branches of an artery in the 16-day-old-CAMs microvasculature and the formation of new pillars was principally observed in those regions where blood flow was enhanced. Using this and many other experiments the group of **Nenad Filipovic** will simulate blood flow through blood vessels and provide necessary data to analyze the distribution of shear stress, as well as fluid pressure and velocity. The software for numerical simulations of fluid flow using a continuum based finite element method was developed in Center for Bioengineering at Faculty of Engineering (Kojic et al., 2008a; Kojic et al., 2008b) and was validated using the analytical solution for shear stress and velocities through a straight expanding tube (**Filipovic** et al., 2003). The alternative to the described continuum-based model is a discretized approach, using discrete methods, such as discrete particle dynamics (DPD) or lattice Boltzmann method (LBM). These discrete methods will also be applied if needed, to obtain more accurate results in microcirculation and to compare the results obtained using different approaches.

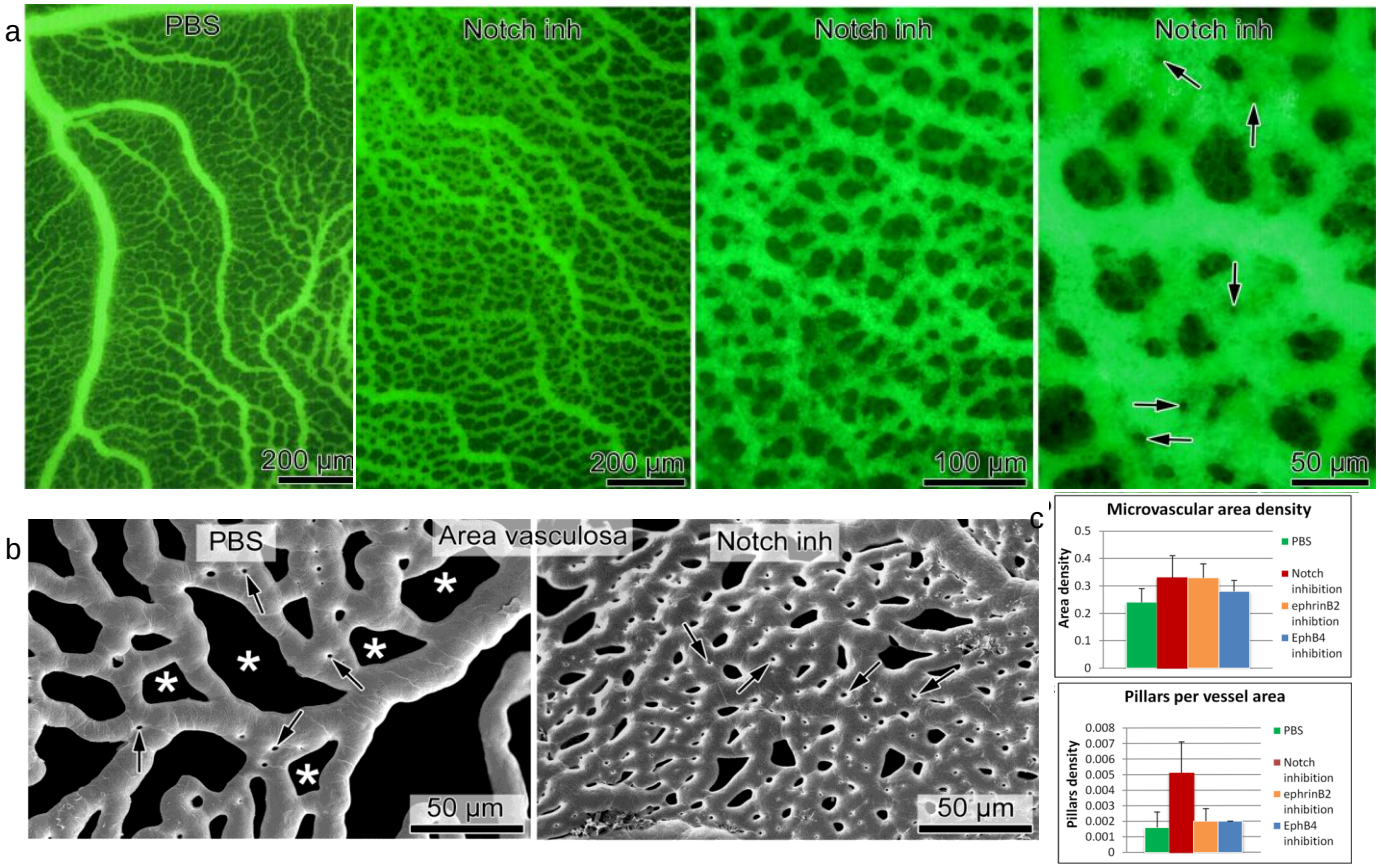

Fig. 1. Inhibition of Notch signalling dramatically induces intussusceptive microvascular growth. (a) and (b) The vasculature of chick area vasculosa 24 h after different inhibitory treatments visualized by FITC Dextran injection. Notch inhibition represented by different magnifications demonstrated very dense capillary meshwork y means of intussusception. (c) Microvascular area density increases considerably after Notch inhibition. Pillar density estimation demonstrated dramatic augmentation primarily after Notch suppression; arrows mark the pillars.

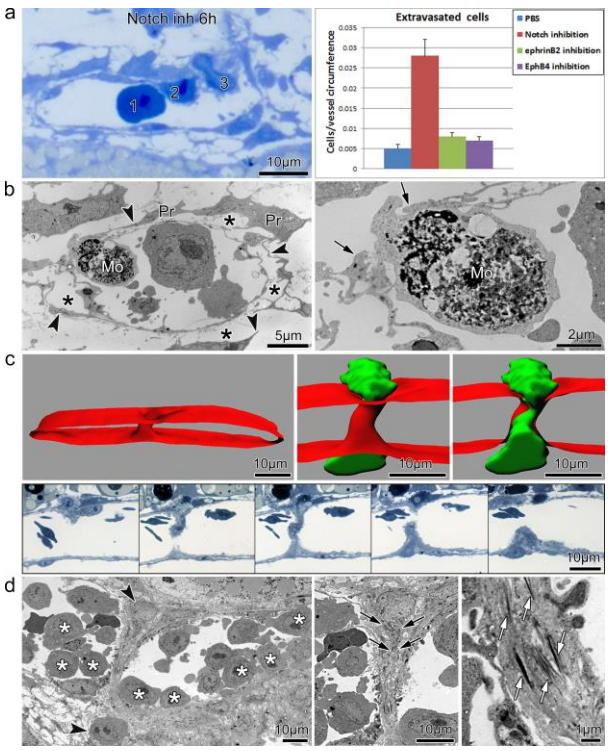

Fig. 2. Extravasation of mononuclear cells after pericyte detachment—contribution to pillar formation. (a) Different stages of mononuclear cell extravasation 6 h after GSI treatment of area vasculosa: attachment to the endothelium (1 and 2), para- or intracellular passage through the endothelium with invasion of the basement membrane (3). The number of extravasated mononuclear cells was about 6 times higher in Notch-inhibited samples as compared to controls. (b) Transmission electron micrographs documented the significantly enlarged periendothelial space treated (asterisks) bounded by the detached pericytes (Pr) and their protrusions. Extravasating mononuclear cell (Mo) passing through the endothelial gap indicated by arrows. Right micrograph is a part of the left at a higher magnification. (c) Pillar reconstruction (3D) of serial semithin sections from GSI-area vasculosa (most important sections demonstrated below) revealing the extravasated mononuclear cells (green) in the vicinity of pillars; the vascular wall is indicated in red. (d) Ultrathin sections revealed cellular protrusions (double arrows) in the vicinity of the pillar and are most likely responsible for the collagen deposition (open arrows) which contributes to additional stabilization of the pillar and finally resistance to flow. Middle and right micrographs are parts of the left one at a higher magnification

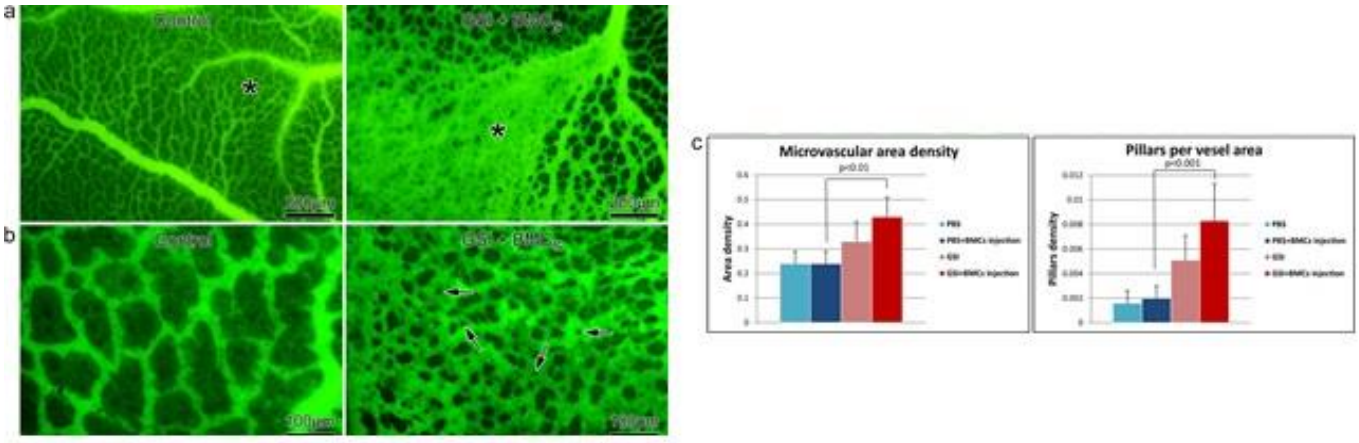

Fig. 3. Injection of BMD cels in the area vasculosa after Notch inhibition (application site indicated by asterisk) dramatically induces increase in microvascular density by onset of IA (arrows, b is higher magnification of a). The area surrounding the application site represented normal vascular pattern similar to the one observed in the controls. c Microvascular area density increases significantly by 80 % after injection of BMD cells in Notch-inhibited samples in comparison with injection of BMD cells in PBS (p\0.01, n = 6). Pillar density demonstrated dramatic augmentation by 63 % compared to the Notch inhibition alone and more than 400 % as compared to PBS (p\0.001, n = 6)

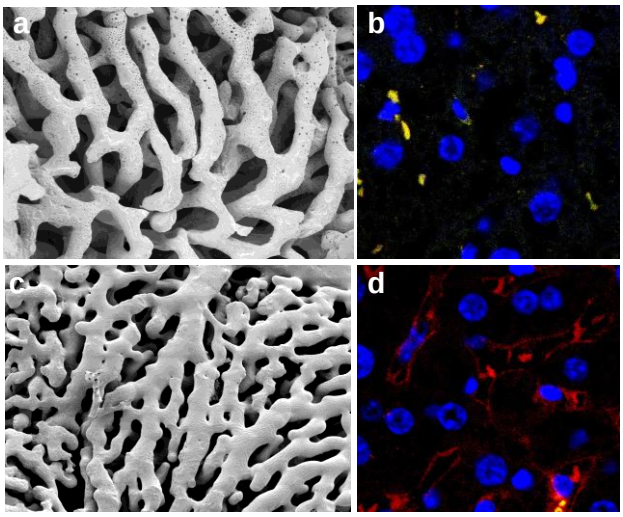

Fig. 4. Vascular casts revealed predominant mode of intussusceptive angiogenesis in liver nodular regeneration after Notch1 knockout (c) compared to wild type mouse (a); the arrows show pillars. Immunofluorescence for SDF-1 demonstrated strong sinusoidal positivity for this marker only in Notch1 knockout mouse (d) but not in the wild type (b).

Center for Bioengineering at Faculty of Engineering, University of Kragujevac, already performed similar analysis using experimental data obtained from mouse colon (**Filipovic** et al., 2009). Fig. 5 shows some of the obtained results. Figure 5A shows the experimental images during the process of intussusceptive angiogenesis and Figures 5B,C, and D show the obtained spatial distribution of velocity and shear stress (**Lee** et al 2010).

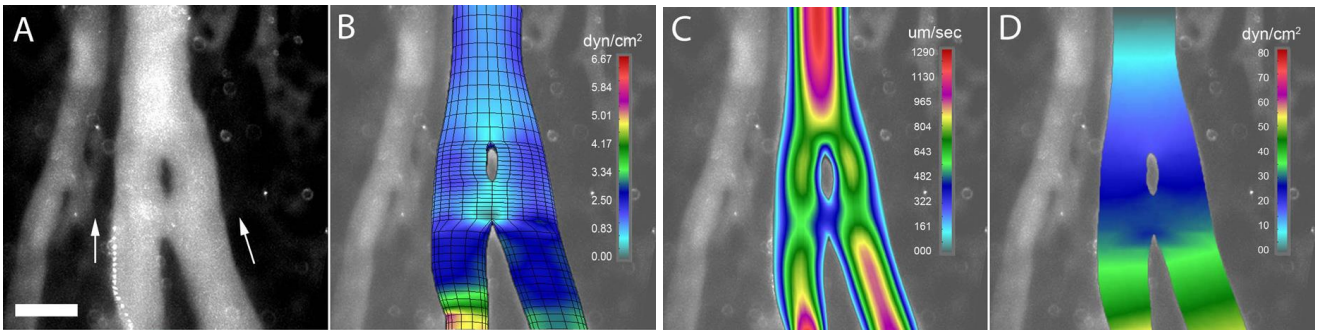

Fig. 5. 3D computational flow modeling of an acute angle bifurcation (5 degrees) in the CAM (CAM (chorioallantoic membrane), (Lee et al 2009).

The research field of group led by **Vladislav Volarevic** is investigation of molecular and cytokine pathways involved in macrophage, T/NKT and dendritic cell mediated pathology. They established the role of IL-33/ST2 signaling pathway in T/NKT cell mediated liver injury (**Volarevic V** et al, 2012) and experimental autoimmune encephalomyelitis (Milovanovic M et al, 2012) and emphasized the role of IL-33/ST2 axis in autoimmune and malignant disease (reviewed in Milovanovic et al, 2012). They were first to show that galectin-3 plays an important pro-inflammatory role in several autoimmune and malignant diseases by promoting the activation and migration of T lymphocytes, NKT cells and DCs, secretion of proinflammatory cytokines, down-regulating M2 macrophage polarization and apoptosis of mononuclear cells (**Volarevic V** et al, 2012, Radosavljevic G et al, 2012) and that mesenchymal stem cells have immunomodulatory characteristics (**Volarevic V** et al, 2011, **Volarevic V** et al, 2013 and **Volarevic et al**, 2011) creating immunosuppressive environment and promote breast cancer in mice through interaction with T regulatory cells, CD8+ T and NK cells (Ljujic B et al, 2013).

All techniques that will be needed to determine the effect of different mononuclear cells on intussusception (determination of phenotype/activation/maturation status and cytokine/chemokine production of tumor infiltrating mononuclear cells) such as flow cytometry, real-time PCR, western blotting, ELISA, immunohistochemistry, immunofluorescence, cell sorting, passive transfer experiments have already been performed and majority of tools (monoclonal and depletion antibodies, primers, tetramers, ELISA sets) are already supplied in Centre for Molecular Medicine and Stem Cell Research University of Kragujevac. At this point of view, there are not any potential obstacles for successful realization of this project.

2.3. SIGNIFICANCE OF THE PLANNED RESEARCH FOR THE SCIENTIFIC COMMUNITY

The group of Valentin Djonov has added significant findings to the field of angiogenesis with particular emphasis on intussusceptive vascular growth, its specific morphological characteristics and its involvement in early development and tumors as such to the current state of the art. **In this project there is now a unique opportunity to build upon that and make significant progress towards the new knowledge for its cellular and molecular mechanism**. CELLS IN ANG represents an innovative, perspective work associated with ambitious tasks.

CELLS IN ANG will endeavour to access the specific populations of bone marrow-derived/peripheral blood cells. CELLS IN ANG recognizes the biologic potential of these cells and aims to define their role in the mechanism of intussusceptive angiogenesis. Although the recent evidence suggests that there is a selection and migration of pro-angiogenic BMD cells towards angiogenic sites (i.e. tissue injury and tumors) there is currently no consistent data about the mechanism of their angiogenic activity and no direct comparison of angiogenic properties for different subpopulations of cells. Moreover, the exact role of the main chemokine player SDF-1/CXCR4 pathway in angiogenesis is still elusive. CELLS IN ANG will use the highly potent models of in vivo angiogenesis (chicken extraembryonic vasculature and zebrafish) along with mouse models and perform comprehensive analysis for the role of BM cells and crucial for their migration and homing signalling SDF-1/CXCR4 in the mechanism of intussusceptive angiogenesis. To our knowledge this is the first study addressing this important research topic. It will be the first mechanistically performed analysis that will provide us with two important types of information: 1) the potential of SDF-1/CXCR4 signaling to induce intussusceptive angiogenesis and 2) how specific cell subpopulations participate in this angiogenic mode. Present data from analysis of monocytes recruitment to ischemic tissues suggest that it can occur without incorporation or differentiation into vascular lineages, assuming their important role in neovascularization by some alternative mechanism of angiogenesis [57-58]. Morphological analysis of samples will represent important tool to prove the occurrence of intussusceptive angiogenesis with respect to BMD/PB cells and SDF-1/CXCR4 signaling. In conjunction with the molecular-biological analyses **CELLS IN ANG will enable us to compare phenotype and function of bone marrow-derived/peripheral blood cells in regard to intussusception and provide new biologic information on pro-angiogenic characterization of these cells**.

Only recently the bone marrow-derived monocytes have been related to VEGF-independent angiogenesis [Botta C et al 2013, Cancer Biol Ther]. The analysis proposed by CELLS IN ANG may be far more explanatory. The members of Djonov's group are among few researchers worldwide with the capacity for analysis of **intussusceptive angiogenesis**. The special and unique spectrum of sophisticated technologies employed by the group is especially suited for the detailed morphological/cellular analysis of the angiogenic mode. We will perform this analysis concomitantly in

chicken area vasculosa, zebrafish and mouse model, and will analyse and reveal distinct characteristics in both cellular and molecular level of this special angiogenic growth. It will allow to actually identifying the specific fraction of cells exhibiting pillar formation capacity, which represent the hallmark of intussusception. A very important strength is, therefore, combining design of the study with technical possibilities in order to perform this challenging analysis, which may prove as a powerful tool for understanding of mechanisms of intussusceptive angiogenesis. Moreover, taking advantage of the possibility to modulate SDF-1/CXCR4 signaling, **CELLS IN ANG will generate new information on possible mechanisms of interference on intussusception through the modulation of chemokine pathways**.

All aspects of CELLS IN ANG work will have the potential to develop towards targeted therapies. Understanding VEGF-independent mechanisms of angiogenesis could aid to explain why currently available anti-angiogenic therapy by Bevacizumab still represents a low- or non-effective therapy. The better insight in the angiogenic process provides the basis for the identification of potential new drug targets taking into account, that CELLS IN ANG will perform a very detailed morphological and phenotypic analysis of the bone marrow-derived/peripheral blood cells in regard to intussusceptive angiogenesis. The ultimate goal of this effort will be to provide the basis for the design of very novel therapies.

As already indicated above, **the research of specific interactions of bone marrow-derived/peripheral blood cells with intussusceptive vascular growth is very novel**, and will have the potential not only to establish for the first time which cells play an important role in mechanism of intussusception and essentially contribute to development of pillars, but also to intervene with these specific interactions and evaluate novel therapeutic targets.

Using the previous knowledge and experimental findings, the assumption was made that intussusceptive angiogenesis is influenced by the appropriate changes in mechanical forces derived from the blood flow. Video recordings and other relevant data about blood vessels in a field of view will be gathered during experiments. Then the off-line analysis with special software will be performed to obtain the geometry of these vessels. Using the numerical simulation software all relevant hemodynamic parameters will be calculated. This will allow us to gain valuable quantitative information and enable a detailed investigation of the dependance of intussusceptive vessel growth of the blood flow. Also, performing several simulations with variable pillar diameter, it is possible to analyze the changes in shear stress distributions and to draw additional conclusions that could potentially help in the regulation of the process of pillar extension.

The outcomes of the CELLS IN ANG as a whole may strengthen the association between the previously published findings, enabling to:

[1] Establish a better understanding for the role of bone marrow-derived mononuclear cells in angiogenesis, in particular intussusception.

[2] Identify the next steps towards the development of better therapeutic strategies.

[3] Define the relationship between hemodynamic parameters and the structural changes in the process of intussusceptive angiogenesis.

2.4. DETAILED RESEARCH PLAN

The first part (**Aim 1**) of our project will deal with the property of SDF-1/CXCR4 signaling to induce and participate in intussusceptive angiogenesis and the potential to influence angiogenic growth by manipulating this pathway. The second part (**Aim 2**) will try to elucidate the contribution of different fractions of mononuclear cells for intussusceptive angiogenesis, using liver model of intussusceptive angiogenesis with SDF-1 positive endothelium. Impact of SDF-1 and mononuclear cells on tumor angiogenesis will be investigated as well (**Aim 3**). The **Aim 4** is dealing with computational investigations of shear stress/flow in intussusceptive angiogenesis.

Aim 1. **To study the role of SDF-1/CXCR4 signaling in intussusceptive angiogenesis**

Preliminary data & rationale: First we aim to show that SDF-1 induces intussusceptive angiogenesis by recruitment of mononuclear cells. We recently generated results for induction of intussusception after injection of bone marrow derived mononuclear cells in Notch inhibited samples of chicken area vasculosa (**Dimova I** et al, 2013). This was associated with an increase in the expression levels of

chemotaxis factors SDF-1 and CXCR4. Pharmacological and genetic manipulations of this pathway seems a good combination of approaches which could be used for prove of its involvement in intussusceptive angiogenesis.

Aim 1a. To investigate vascular morphogenesis in chicken area vasculosa upon stimulating and inhibiting SDF1/CXCR4 signaling

Hypothesis: *Administration of SDF-1 could support revascularization of ischemic tissues by means of intussusception, whereas inhibition of CXCR4 may provide an effective means to block intussusceptive angiogenesis*.

Rationale: SDF-1–positive endothelium was found lining the newly formed intraluminal vessels in lobular capillary hemangiomas (Morrow D et al, 2007), possibly these were sites of pillar formation. Wrag et al. demonstrated that transplantation of rat bone marrow-derived progenitor cells, positive for VEGFR1 and CXCR4, in ischemic hind limbs increased capillary density by a SDF-1 dependent manner, but did not differentiate into vascular structures like endothelial cells or smooth muscle cells (Williams CK et al, 2008). In our previous study, we observed up-regulation of SDF-1 and CXCR4 after Notch inhibition being in association with intussusceptive angiogenesis. The latter factors are most probably essential for the recruitment of mononuclear cells, participating in the formation of pillars. Thus, ***inhibition of CXCR4 may provide an effective means to block intussusceptive angiogenesis, whereas administration of SDF-1 could support revascularization of ischemic tissues by means of intussusception***.

Aim 1b. To study the formation of caudal vein plexus in zebrafish with depleted SDF-1

Hypothesis: *Depletion of SDF-1 by morpholino could affect the formation of caudal vein plexus, which is normally formed by means of intussusception*.

Rationale: Recently Djonov V. et al have clearly demonstrated that the honeycomb-like vascular network in zebrafish, which is ventral expansion of the axial vein and is termed caudal vein plexus (CVP), is formed and organized by intussusception, since the appearance of characteristic pillars was live-imaged and monitored (data not published). Surprisingly, an increasing numbers of round cells with the morphology of hematopoietic precursors or lymphocytes (scant cytoplasm and a round nucleus with condensed clumps of chromatin) were noticed in this area (Murayama E et al, 2006). This hematopoietic population paralleled the development of the CV plexus and its further evolution into a single caudal vein. In addition, SDF-1 expression was detected by in situ hybridization in exactly the same region by Walters et al (2010). We plan ***to investigate how the caudal vein plexus will be formed after depletion of SDF-1*** by morpholino in zebrafish embryo.

Experimental design: The chicken area vasculosa will be inhibited by GSI which induces intussusception as it is described in our previous publication (Dimova I. et al, 2013). Three groups will be investigated: in the first group CXCR4 will be blocked by AMD3100 together with Noth inhibition (=GSI+AMD3100), the second group will be treated only with AMD3100 (no GSI) and the third group will remain untreated. Recombinant SDF-1 protein will be applied to stimulate the signaling in vivo. The protocol is already established in the lab. The methodology for vessel investigation in zebrafish is well established in the lab of V. Djonov (Hlushchuk R et al, Nat Methods, submitted for publication). SDF-1 depletion will be achieved by injection of morpholino into the yolk of 1- to 2-cell–staged embryos as it is described in the literature (Walters et al 2010).

End points & methodological approaches: All available methods for investigation of permeability, extravasated cells, morphology and intussusception in chicken area vasculosa will be applied - in vivo microscopy, serial semithin sectioning, immunohistochemistry (IHC), immunofluorescence (IF), transmission electron microscopy (TEM), scanning electron microscopy (SEM). The efficacy of inhibition/overexpression will be evaluated by real-time RT-PCR as well as by immunoblotting. We will determine vascular morphogenesis in chicken area vasculosa under treatm ent by SDF-1 and upon inhibition of SDF-1/CXCR4 signaling by AMD3100; establish the angiogenic mode during the formation of caudal vein plexus in zebrafish with depleted SDF-1. The essential quantitative parameters such as vascular density and pillar number per vessel area will be routinely done, as they are described by Djonov group (**Makanya A** et al, 2007). Tie2-gfp zebrafish up to 72 hpf (hours post fertilization) will be used for the monitoring of vessel formation after SDF-1 depletion by morpholino using an

epifluorescence microscope (Polyvar-Reichert, Glattbrugg, Switzerland) equipped with a Canon 5D Mark II camera for both video recording and taking of still images.

Aim 2. **To study the role of different BMD and PB cells for intussusceptive angiogenesis in liver model with endothelial positivity for SDF-1**

Preliminary data & rationale: In a recent investigation of the group Djonov (Dimova I. et al, data not published), expression of SDF-1 was detected in sinusoidal endothelium of Notch1 KO mouse, which was previously shown to increase intussusceptive microvascular growth in the liver (Dill et al, 2012). The expression of SDF-1 exerted dynamical changes with the time. By day 8 its expression was already extensively spread on all sinusoids with high intensity. In comparison, wild type mouse liver didn't present any positivity for SDF-1 expression at the same time point. These results suggest that vessel-derived SDF-1 may be responsible for intussusceptive vascular remodeling in the liver of Notch1 KO mouse.

Since it is well known that blocking of SDF-1/CXCR4 axis results in prevention or delay of tumor recurrence after irradiation by inhibiting the recruitment of CD11b+ monocytes/macrophages that participate in tumor revascularization (Tseng D et al, 2011), **we will examine the presence and the role of macrophages in the process of intussusceptive angiogenesis.** Since it was recently shown that blockade of CXCR4 and iNOS is able to inhibit lung metastases in a xenotransplanted mouse model of adenoid cystic carcinoma of the oral floor (Takaoka K et al, 2013), it will be interesting **to determine the interplay between neutrophils, NO and SDF-1/CXCR4 axis during the process of intussusceptive angiogenesis**. It is known that CXCR4 is expressed on eosinophils (Dulkys Y et al, 2004) and concentrations of SDF-1 correlates with eosinophil recruitment (Negrete-García MC et al, 2010), t**hus one of aims of this study is to determine the effects of SDF-1 on recruitment and activation of eosinophils during intussusceptive angiogenesis.** Since it is known that SDF-1/CXCR4 signaling has pivotal role in mast cell (MC) recruitment in tumor tissue (Polajeva J et al, 2011) and that MC produce pro-angiogenic chemokines in response to SDF-1 (Lin TJ et al, 2001), in this study we are going **to investigate the role of SDF-1/CXCR4 axis on mast cell recruitment in intussusceptive angiogenesis**. As CXCR4+ dendritic cells (DC) promote angiogenesis during embryo implantation in mice (Barrientos G et al 2013) and CXCR4 is known as critical chemokine receptor for migration of plasmacytoid DC (Umemoto E et al, 2012), **it will be of particular interest to determine the impact of SDF-1/CXCR4 axis on activity of DC during the process of intussusceptive angiogenesis.** Although it is well known that CXCR4 is expressed on both NK and NKT cells and regulates their migration in inflamed and tumor tissues in response to SDF-1 (Robertson MJ, 2002 and Lindau D, 2013), **the role of SDF-1/SCXR4 axis in NK/NKT cell mediated angiogenesis is unknown and will be investigated during this study.** SDF-1/CXCR4 signaling is important for migration and activation of T cells (Patrussi L et al, 2008). However, **the role of SDF-1/CXCR4 signaling in T cell mediated angiogenesis is unknown and will be determined in this study, particularly its role in the process of intussusceptive angiogenesis**. Since B cells promote tumor progression via STAT-3 regulated-angiogenesis (Yang C et al, 2013) and SDF-1/CXCR4 axis is essential for B-lymphocyte production (reviewed in Nagasawa T et al, 2000) and maintenance of B-cell homeostasis (Mountz JD et al, 2011), **in this study we will explore the role of SDF-1/CXCR4 signaling for B-cell activity in intussusceptive angiogenesis.**

Hypothesis: *Specific BMD and PBM cells may have different contribution to the mechanism of intussusceptive angiogenesis*.

Experimental design: The Notch1 KO model of intussusceptive angiogenesis (Dill et al, 2012) will be used for studying the participation of different bone marrow derived/peripheral blood cells in this angiogenic mode in relation to SDF-1. Material will be collected from mouse liver of Notch1 KO and WT mouse at time points day 2, day 6, day 8 and day 14. For all these days we have vascular casts and quantitative data for intussusceptive angiogenesis. Temporal evolution of the angiogenesis-linked factors (SDF-1/CXCR4; FGF2-R and FGF2; PDGF-B and PDGFRβ; VEGF and VEGF-Rs 1 and 2; HIF -1α) will be evaluated by immunohistochemistry, Western blotting and RT-PCR. Additionally, profile of inflammatory mediators involved in angiogenesis will be determined by ELISA and real-time PCR. Macrophage inflammatory protein (MIP)-1α, monocyte chemotactic protein (MCP)-1, keratinocyte-derived chemokine (CXCL1/KC), VEGF, TNF-alpha, IL-1β, CCL11, bFGF, GM-CSF, PDGF, TGF-β, IL-6, IL-8, CoX2, PDGF-β, MMP7, MMP9, MMP12, IL-10, IFN-γ, IL-12, IL-4, IL-17 and IL-23 will be

measured in sera and liver tissues of experimental mice. The material from KO and WT mouse will be used for the investigation of different cell types and activities. If results obtained *in vivo* show that there is significant difference in cytokine/chemokine profile of macrophages, T lymphocytes and/or NKT cells isolated from KO and WT mice, we will stimulate these cells *in vitro* (by using IFN-gamma (150 U/ml) and LPS (10 ng/ml) for macrophage activation, anti-mouse CD3e, clone 145-2C11 (0.5-0.1 µg/mL), anti-mouse CD28, Clone 37.51 (2 ug/mL) and Concanavalin A (1-4 µg/mL) for stimulation of T cells, α-GalCer (100 ng/ml in 0.1% DMSO) for stimulation of NKT cells) according to manufacturer's instructions and previously published protocols (Tomura M et al, 1999 and Mosser DM et al, 2008) and we will determine the level of cytokines/chemokines (MIP-1α, MCP-1, CXCL1/KC, VEGF, TNF-alpha, IL-1β, CCL11, bFGF, GM-CSF, PDGF, TGF-β, IL-6, IL-8, CoX2, PDGF-β, MMP7, MMP9, MMP12, IL-10, IFN-γ, IL-12, IL-4, IL-17 and IL-23) in supernatants by ELISA test. Also, the impact on NK cell activity on intussusceptive angiogenesis of liver will be evaluated by depletion of NK cells, which will be done *in vivo* by using anti-asialo GM1 antibody. If we find any difference in percentage, absolute number and/ or cytokine profile of liver infiltrating NKT cells in KO and WT mice, we will conduct passive transfer experiments in which sorted NKT cells pooled from KO and WT mice will be adoptively transferred in WT and KO mice, respectively ($1x10^6$ NKT cells/mouse). If we find that SDF-1 modulate DC maturation, phenotype and/or angiogenic activity, we will try to modulate intussusceptive angiogenesis in liver by passive transfer experiments in which sorted DC cells, pooled from KO and WT mice, will be adoptively transferred in WT and KO mice, respectively ($5x10^5$ DC cells/mouse).

End points & methodological approaches: Using the methodology of flow-cytometry, western blot, real-time–PCR, immunofluorescence and immunohistochemistry we will determine: (1) the relative and absolute number of VEGF, bFGF, TNF-alpha, IL-1β, IL-8, CoX2, PDGF-β, MMP7, MMP9 and MMP12-producing CD11b+ and F4/80+ macrophages, expressing activation markers CD80 and CD86; (2) presence of CD66a+, CD66b+, CD11b+ and CD14+ neutrophils and the release of NO, superoxide dismutase, total glutathione and reactive oxygen species from activated neutrophils; (3) eosinophil infiltration in liver by Congo staining, immunohistochemistry (using antibodies recognizing mouse major basic protein) and fluorescence-activated cell sorting for the expression of Siglec-F, F4/80, CD11b, and Gr-1; (4) the percentage and absolute number of bFGF, IL-6, IL-8, GM-CSF, PDGF, TGF-β and MMP9-producing cells in the population of sorted eosinophils; (5) the presence of mast cells in liver by adapted method of Strobel, Miller, and Ferguson as well as basic Giemsa or toluidine blue staining, while flow cytometry will be used for counting VEGF, bFGF, MMP9, TNF-α, TGF-β, CCL2, IL-8 - producing liver infiltrating CD34+ FcεR+ mast cells; (6) the presence of VEGF, CXCL1, CXCL2, CXCL3, CXCL5, TNF-alpha, IL-12, IFN-gamma, IL-1β, IL-6, IL-8- producing $CD11c^+$ dendtritic cells in liver samples, also maturation (expression of CD40, CD80, CD86), regulatory (CD11c+CD8+) and inflammatory phenotype (CD11b+Gr-1+, MHC class II+ and/or CD1d+) of liver infiltrating DC; (7) the percentage and absolute number of TNF-α, IFN-γ, IL-4, IL-17 and IL-10 producing NK1.1+ NK cells; (8) the relative and absolute number of TNF-alpha, IFN-gamma, IL-4, IL-10, IL-17- producing CD4+ T-cells, IFN-gamma-producing CD8+CTLs, TGF-beta, IL-10 and VEGF-producing CD4+CD25+Foxp3+ T-cells; (9) the infiltration of STAT-3+ CD19+ B cells.

Aim 3. **<u>To study the role of SDF-1 and mononuclear cells for tumor angiogenesis and tumor growth</u>**

Preliminary data & rationale: In a recent investigation of the group of Djonov (Hlushchuk R et al, 2008), mice bearing xenogratfts of MMTV/c-neu mammary adenocarcinomas underwent either radiotherapy or treatment with the VEGF-receptor tyrosine-kinase inhibitor PTK787 (Novartis Pharma). The post-treatment period was characterized by several key events, which together acted as an escape mechanism: tumour recovery after radiotherapy is associated with intussusceptive microvascular growth. Quantification of the evaluation of the newly-formed pillars after either irradiation or treatment with PTK 787 revealed an angiogenic switch from sprouting to intussusception.

Experimental design: The tumor model of intussusceptive angiogenesis (Hlushchuk R et al, 2008) will be used for studying the expression of SDF-1 after different treatment and together with intussusceptive angiogenesis. In treated and non-treated groups TAMRA-labeled bone marrow-derived mononuclear cells, identified as crucial from the Aim 2, will be injected intravenously before treatment and their localization will be observed after sacrificing the mice on days 5, 9 and 14 after the onset of treatment.

End points & methodological approaches: Temporal evolution of the angiogenesis-linked factors (SDF-1/CXCR4; FGF2-R and FGF2; PDGF-B and PDGFRβ; VEGF and VEGF-Rs 1 and 2; HIF -1α) will be evaluated by immunohistochemistry, Western blotting and RT-PCR in treated and non-treated tumors. All relevant methodologies are routinely implemented in the laboratory. The cells with already defdined implementation in IA from previous Aim will be labeled by TAMRA and injected in mice. Their recruitment will be observed by fluorescent microscopy after sacrificing the mice on days 5, 9 and 14 after the onset of treatment.

Aim 4: <u>To perform numerical simulations and calculate shear stress distribution</u>

Short description: The group of Djonov performed experiments in which they proved that mechanical forces induced by blood flow when one of the branches of the artery is blocked lead to the increase of pillar formation in the remaining regions. Thus, there is a connection between the type of flow through the vessels (laminar, turbulent, oscillatory, pulsatile, steady) and the intensity of mechanical forces with gene expression and further with pillar formation. But this connection is not entirely analyzed and here numerical simulations can help providing additional useful information about blood flow.

Rationale: In order to provide a detailed analysis of the spatial distribution of physiologic forces and shear stress, a 3D computational model of the intraluminal intussusceptive pillar will be developed. The simulations will be performed using a continuum based model. The fluid flow will be modelled using finite element method. The system of equations that is solved is given by:

$$(1)\ \rho\left(\frac{\partial v_i}{\partial t}+v_j\frac{\partial vi}{\partial x_j}\right)=-\frac{\partial p}{\partial x_i}+\mu\left(\frac{\partial^2 v_i}{\partial x_j\partial x_j}+\frac{\partial^2 v_j}{\partial x_j\partial x_i}\right) \qquad (2)\ \frac{\partial v_i}{\partial x_i}=0$$

where the summation is assumed on the repeated (dummy) indices (i,j=1,2,3). The first equation is the Navier-Stokes equation, that represents the balance of linear momentum, and the second equation is the continuity equation, that ensures the incompressibility condition. In both equations blood velocity in direction x_i is denoted by v_i, fluid density is denoted by ρ and dynamic viscosity is denoted by μ.

Wall shear stress actually represents the viscous force that is acting tangentially on the vessel wall. In numerical simulations wall shear stress is calculated as: $\vec{\tau}=-\mu\frac{\partial\vec{v}_t}{\partial\vec{n}}$ (3) where $\vec{v}_t$ represents the tangential velocity and $\vec{n}$ is the normal direction at the vessel wall. First the tangential velocity is calculated at the mesh points near the wall and then the velocity gradient is evaluated using the calculated quantities. Finally, three components of the wall shear stress vector are obtained (τ_x,τ_y,τ_z). The effective value of wall shear stress at the finite element mesh nodes can be calculated as:

$$\tau_{eff}=\sqrt{\tau_x^2+\tau_y^2+\tau_z^2} \qquad (4)$$

Using experimental data the geometry for the computational model will be created. The obtained fields of blood velocity, pressure and shear stress will be compared with the observed vascular morphological changes, to determine more details about the connection between these two phenomena.

2.5. INDIVIDUAL TASKS AND RESPONSIBILITIES

Milestone	Associated activity	Expected duration	Responsible project partner
1. Determine vascular morphogenesis in chicken area vasculosa under treatment by SDF-1	RTD, Dissemination	1 - 6 months	VD, ID
2. Determine vascular morphogenesis in chicken area vasculosa upon inhibition of SDF-1/CXCR4 signaling by AMD3100	RTD, Dissemination	1 - 6 months	VD, ID
3. Establish the formation of caudal vein plexus in zebrafish with depleted SDF-1	RTD, Dissemination	1 - 12 months	VD, ID
4. Determine the SDF-1 endothelial expression and its correlation with intussusception in the mouse model	RTD, Dissemination	1 - 36 months	VD, ID
5. Determine macrophage activity in the process of intussusceptive angiogenesis of liver	RTD, Dissemination	12 – 24 months	VD, VV
6. Determine the role of neutrophils in intussusceptive angiogenesis of liver	RTD, Dissemination	12 – 24 months	VD, VV
7. Determine eosinophil infiltration in liver with intussusceptive angiogenesis	RTD, Dissemination	12 – 24 months	VD, VV
8. Determine the presence of mast cells in liver with intussusception	RTD, Dissemination	12 – 24 months	VD, VV
9. Investigate migration and activation of dendritic cells (DC) in the process of intussusceptive angiogenesis of liver	RTD, Dissemination	24 – 36 months	VD, VV
10. Analyze the percentage and absolute number of IFN-gamma and TNF alpha producing NK1.1+ NK cells in liver	RTD, Dissemination	24 – 36 months	VD, VV
11. Determine T cell activity in the process of intussusceptive angiogenesis of liver	RTD, Dissemination	24 – 36 months	VD, VV
12. Analyze the infiltration of STAT-3+ CD19+ B cells in liver samples with intussusceptive angiogenesis	RTD, Dissemination	24 – 36 months	VD, VV
13. Definition of geometrical model for intraluminal intussusceptive pillar	RTD, Dissemination	1 - 6 months	NF
14. Definition of boundary conditions of the intraluminal intussusceptive pillar	RTD, Dissemination	6 - 12 months	VD, NF
15. Results for 3D computational model of the intraluminal intussusceptive pillar and comparison with experiments	RTD, Dissemination	12 - 36 months	VD, ID, VV, NF

VD – Valentin Djonov; ID – Ivanka Dimova; VV – Vladislav Volarevic, NF – Nenad Filipovic

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3. TRANSITION RELEVANCE OF THE PLANNED RESEARCH (max. 5 pages)

3.1 SIGNIFICANCE OF THE PLANNED RESEARCH FOR THE SUSTAINABLE ACADEMIC, ECONOMICAL AND SOCIETAL DEVELOPMENT OF THE PARTNER COUNTRY

Revealing the molecular nature of angiogenesis engages the attention of the scientific community for many years and due to the extreme complexity of the process it will raise questions and scientific discussions still for a long time in the future. The team wishes to continue the traditions and work on this current essential issue. We believe that the results will be very interesting and useful to future scientific, clinical and pharmaceutical research and that will create opportunities for continued research after the finalization of the project.

For carrying out the purposes of the project it is essential to combine knowledge from different disciplines - molecular biology, immunology, biochemistry, pharmacology, morphology, cell biology, imaging, computer sciences and others. Collaboration with the experts in the relevant fields will enrich the knowledge and experience of the partners and would allow subsequent realization of research with real practical application.

The possibility for further research on the subject and after the project is a good basis for further expansion of the contacts of the partners with research institutions at home and abroad. Knowledge and valuable experimental material increase chances of participation in consortia of major projects under EU programs.

The project brings together highly skilled and complementary researchers from both basic and clinical science backgrounds. Indeed, the members from the Host organization are world leaders in the field of intussusceptive angiogenesis and morphological research. The proposed project will synergize the efforts and facilitate integration of research capabilities in order to increase coherence and critical mass in the study of molecular and cellular mechanisms of intussusceptive angiogenesis. In meeting the scientific and technological objectives of the current research project, a multidisciplinary approach is taken which maximizes the potential to provide crucial insights into the basics of angiogenesis.

Holders of knowledge in the project (coordinator and partners) are highly motivated to share knowledge in the field of intussusceptive angiogenesis and to monitor the transfer of knowledge to young researchers. Both the Coordinator and the partners, have extensive interactions with key stakeholders, including the lay public, patients, students, as well as academic and commercial parties, which will be built further upon the present proposal. In the knowledge transfer will be included conventional means such as publications, reports of meetings and preparation of projects, organization of seminars where the researchers will be able to present their work, to discuss problems and future strategy and to seek the opinion of experts.

If the results of the study identify with suitable accuracy that SDF-1/CXCR4 and specific phenotype of BMD cells are responsible for intussusceptive angiogenesis, we would like to move towards the development of appropriate therapeutic strategy to enter further investigations. The project results will be very useful for future developments related to the search of target molecules and new pro- and anti-angiogenic therapy. This subject is of particular interest to pharmaceutical companies these days and successful identification of informative cellular/molecular targets predict collaboration and access for project results to stakeholders with commercial interests (such as pharmaceutical companies).

The acquired new skills and competences will give rise to high quality research for the partners, based on integrated molecular-biological and histo-morphological knowledge.

1. Transfer of knowledge in vascular biology and development - to work for the development of highly qualitative research in their country.

Upon the project, they will be able to serve their universities for development of molecular vascular biology, immunology, immunohistochemistry, proteomics and functional genomics laboratory and expand their attainment to their MD and master’s students. Bulgaria is a new member of EC, and the government has been prioritizing the goal for molecular and biotechnological development, as a significant and urgent co-requisite for the country health care improvement in the terms of prophylaxis and predictive medicine. Through expected scientific achievement from University of Bern SCOPES program, the partners will have a unique level of achievement in advanced learning, research and scientific innovation about vascular biology and development.

2. Elaboration of new research projects

The earned knowledge and skills through University of Bern in vascular biology and development will largely be utilized for research development in the institution the partners work for. They will be able to transfer their education consequently to research projects and establish center of excellence regarding molecular vascular biology and development with molecular-biological, immunological, proteomic and functional genomic research labs. Moreover their expected excellence will also be rendered for the demand driven technology innovation and research for the local and international human health.

3. Development of educational programs and Problem Based Learning (PBL) in the Institution they work for.

4. Establish in a long term a collaboration with exchange of future PhDs and master students.

Following the good experience of Prof. Djonov who established ERASMUS program from University of Bern and Fribourg, SCOPES program will be the way for establishing a partnership in this program from the Medical University of Sofia and University of Kragujevac.

3.2 PLANNED ACTIONS TO STRENGTHEN RESEARCH CAPACITIES IN THE PARTNER COUNTRIES

The methods planned to be applied in the study are very new and advanced. The fact that team laboratories are skilled to perform them, gives a unique opportunity to the other members of the consortium to be trained in them. If the project is selected, **mutual training courses** will be organized with the aim to exchange experience and to improve technical skills. **Exchange of researchers** in the limits of the project is also planned to take place. These researchers would be given an opportunity to make themselves familiar with new technologies working on the project objectives in the mean time.

The models for studying intussusceptive angiogenesis have not been introduced in Serbia and Bulgaria so far. The familiarizing with these latest technologies of the Bulgarian and Serbian teams would help their **implementation in the region** which will lead to improved basic and translational research in the field of angiogenesis.

Strengthen the position of the young researchers by a cycle of special Information Days which promote the achievements of the young scientists in accomplishing of the project. The research group will organize international course for training of young scientists from involved Balkan countries. Undergraduate, PhD and postdoctoral students will participate in the project execution.

International workshops and seminars This activity includes selection of junior scientists for participation in advanced training courses and appropriate workshops organised by international institutions for functional genomics. These institutions will contribute to the project by disseminating knowledge in the area of angiogenesis research and by providing young scientists fellowships.

As a part of our project we aimed to organize a **Balkan Summer School*:*** “Cellular and molecular mechanisms of intussusceptive angiogenesis”**.** Aims:

To create highly trained physician/scientists who will establish careers in cellular and molecular biology, who are able to successfully compete for European grants, and who will conduct “state of the art” basic and translational research directly related to angiogenesis.

To acquire intellectual skills: reason critically; analyze and interpret scientific data; solve problems; apply knowledge to research project. The best participants will be stimulated to prepare proposals for participation in the new European grants which support individual ideas to be realized with own team. This is an ideal opportunity for the development of the human potential.

*To acquire practical skills***:** design and execute experiments; analyse experimental data; draw qualitative and quantitative conclusions from available data and the discernment as to whether such conclusions are justified; retrieve, sift and select information from a variety of sources.

To acquire transferable skills: structure and communicate ideas effectively both orally and in writing; manage time and work to deadlines; participate in discussion groups; work independently and be self-reliant; work as a member of a team; solve research problems; solve numerical problems; find information and use information technology; prepare and present a poster; present a research talk.

Focus of the Summer School is also to improve the communication between experts and students interested in different aspects of angiogenesis. It will furthermore allow doctoral students and young post-docs to develop contacts to experts in their fields but also to workers that focus on different aspects of this process.

3.3 STRATEGY TO COMMUNICATE WITH INTENDED RESULT USERS

3.3.1. OVERALL STRATEGY

The standard strategy developed for implementation of projects will be followed.

The expected results concern all laboratories dealing with angiogenesis. They will use the project outputs in the detection of cellular/molecular markers associated with intussusceptive angiogenesis, its involvement in diseases and treatment strategies.

The expected results also concern all oncologists and cardiologists dealing with neo-angiogenesis. They will use the project deliverables in their practice for stimulating or suppressing angiogenic growth.

The results from the scientific research will benefit the patients since they could help to define markers for specific angiogenic growth, to conduct related diagnostic activities and to improve the therapy connected to angiogenesis.

The end-users of the project outcomes are:

- Other academics working in the field of angiogenesis
- Enterprises developing therapeutic service
- Other research groups involved with oncology and cardiology

Our dissemination objectives are:

- Wide awareness of the project among relevant academics, end-users and policy customers
- Direct transfer of knowledge to other relevant research programmes and to potential end-users
- Open and transparent engagement of the academic, and user communities (together with relevant policy makers) in any ethical and societal issues

3.3.2. A DETAILED DESCRIPTION OF THE DISSEMINATION PLAN:

a) Publications in peer review journals

The results of the joint research will be published in peer reviewed international journals.

b) Organization of workshops, seminars, posters sessions for students and young researchers

International workshops, poster sessions for students and young researchers from different countries and seminars on the project topics will be organized.

The results from the research studies will be presented at EU scientific conferences.

Cycle of special thematic Information Days will be performed.

c) Balkan Summer School

A Balkan Summer School “Cellular and molecular mechanisms of intussusceptive angiogenesis” will be carried out as above mentioned. The best participants will be stimulated to prepare proposals for participation in the new European grants which support individual ideas to be realized with own team. This is an ideal opportunity for the development of the human potential.

Focus of the Summer School is to improve the communication between experts and students interested in different aspects of genetic problems of common diseases. It will furthermore allow doctoral students and young post-docs to develop contacts to experts in their fields but also to workers that focus on different aspects of angiogenesis.

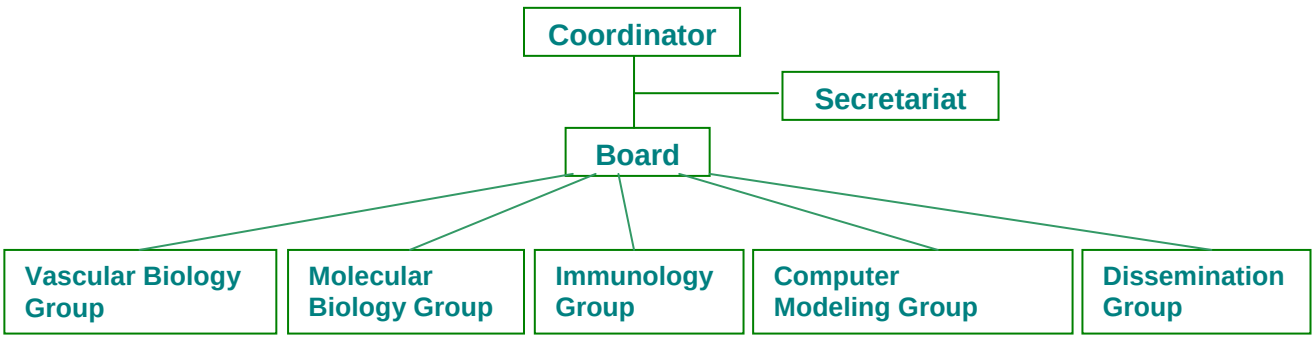
The course is aimed at researchers with a background in cell and molecular biology who wish to gain an insight into the most up-to-date methodologies used in angiogenesis research. The course is at masters-level.

This course aims to give an introduction to the techniques used in vascular biology and to the analysis methods necessary for understanding the role intussusceptive angiogenesis play in biological systems. The course will start by looking at the relationship of molecular signaling to cellular interactions. The principal methods for studying angiogenesis will then be surveyed and the most sophisticated morphological analyses will be introduced.

4. MANAGEMENT STRUCTURE

The complexity involved in achieving the project goals and the level of integration needed between research areas and partners requires a robust and transparent management structure and decision-making process. Figure 1 show the organisational structure planned to works in project execution.

Figure 5. Organizational structure



4.1. The Board

The Board is the central decision making forum for the project. All participants are represented in the board and involved in at least one main task where all technical activities on a day-to-day basis will be managed. The Board will manage and co-ordinate the strategic operation of the tasks to ensure over the life of the project achieves it objectives after 3 years.

The Board is chaired by the co-ordinator Prof Valentin Djonov (University of Bern, Switzerland) and also includes the lead scientist from each partner: Prof. Vladislav Volarevic (Serbia), Assoc. Prof. Ivanka Dimova (Bulgaria). The Board will be ultimately responsible for all key decisions affecting overall project development. The Board will manage the tasks acting as the key channel for monitoring the progress of the tasks in meeting project objectives.

The Board must approve:

1. Deliverables and milestone setting
2. Annual reports
3. Overall co-ordination and knowledge management including planning, carrying out and controlling the shared knowledge
4. Overall legal, contractual, ethical, financial and administrative management issues
5. Promotion of gender equality
6. Strategic planning, monitoring of progress and revisions to the plan
7. Financial Management including decisions related to the budget and resource allocation
8. Co-ordination of RTD, Dissemination, Demonstration and Training activities
9. Planning for common resources, assignment and effective control of resources
10. Communication plan and organisation

As the Board comprises 3 members in total the required quorum will be 2. Board members will be allowed to appoint substitutes for individual meetings they are unable to attend. The Board's decision on all matters will be final. It is anticipated the Board will need to three times per year.

4.2. The Tasks

There are 15 tasks (see List of Activities) including RTD together with separate Management, Dissemination, Training and Demonstration. Five research groups will function in accomplishing tasks' objectives:

- **Vascular Biology Group** (VD/ID): responsible for *Determine vascular morphogenesis in chicken area vasculosa under treatment by SDF-1; Determine vascular morphogenesis in chicken area vasculosa upon inhibition of SDF-1/CXCR4 signaling by AMD3100; Establish the formation of caudal vein plexus in zebrafish with depleted SDF-1;*
- **Molecular Biology Group** (ID): responsible for *Determine the SDF-1 endothelial expression and its correlation with intussusception in mouse liver,*
- **Immunology Group** (VV): responsible for *Determine macrophage activity in the process of intussusceptive angiogenesis of liver; Determine the role of neutrophils in intussusceptive angiogenesis of liver; Determine eosinophil infiltration in liver with intussusceptive angiogenesis; Determine the presence of mast cells in liver with intussusception; Investigate migration and activation of dendritic cells (DC) in the process of intussusceptive angiogenesis of liver; Analyze the percentage and absolute number of IFN-gamma and TNF alpha producing NK1.1+ NK cells in liver; Determine T cell activity in the process of intussusceptive angiogenesis of liver; Analyze the infiltration of STAT-3+ CD19+ B cells in liver samples with intussusceptive angiogenesis;*
- **Computer Modeling Group** (NF): responsible for *Definition of geometrical model for intraluminal intussusceptive pillar; Definition of boundary conditions of the intraluminal intussusceptive pillar; Results for 3D computational model of the intraluminal intussusceptive pillar and comparison with experiments.*
- **Dissemination Group** (VD): responsible for the dissemination of all results generated by the project;

The leaders of the tasks are drawn from the organisations represented at Board level. The responsibilities include:

1. Formulate and conducting the technical aspects of the activity
2. Ensuring individual participants in each activity are compliant with national ethics frameworks and have gained approval from their appropriate institutional ethical committees
3. Reporting progress to the Board

Each activity is lead by representatives of the organisations on the Board and other key individuals elected by the Board where appropriate. Each activity comprises representatives of consortium partners. Membership of each activity will be appointed by the Board but will be based on recommendations of the respective leaders.

It is envisaged each team will agree a programme of meetings at the beginning of each year according to the objectives to be met for that activity. These will occur at least 1-2 times per year.

4.3. Coordinator and Secretariat

The Coordinator leads the Secretariat which is responsible for day-to-day administrative issues. The leaders of each team will assist the Secretariat to monitor the progress in each activity against agreed milestones with an established simple yet effective one-page quarterly reporting procedure.

4.4. Consideration of gender aspects

Consistent with the aims of the Helsinki Group on Women and Science, the consortium will seek to maintain and increase the involvement of women in the programme. The following initiatives will be implemented:

- The consortium will aim to employ an equal proportion of men to women within its research staff with a conscious effort to consider gender issues. The percentage of women involved in the project will be monitored annually and women will be continually encouraged to participate in science at all levels
- Where Board members or Team leaders are unable to attend meetings a female representative will be encouraged to attend in their place
- Activities to increase awareness in science of the importance of gender equality will be undertaken. In addition steps will be made to encourage more women into science promoting flexible working hours and family friendly policies
- Establishment of a Standard Operating Procedure for monitoring gender equalities
- The consortium will adopt a universal gender equality policy which will use a set of indicators to measure the progress of partners in increasing/maintaining levels of gender equality. Indicators will measure recruitment, retention and career development of women as well as progress in compliance with policies, procedures and programmes which affect the position of both men and women

4.5. Ethical considerations

The Swiss Institution has already obtained permission for conducting animal experiments with mice. They are familiar with all rationales for animal usage, including animal transportation methods, type and mode of anesthesia, survival surgery with methods of asepsis, post-operative care, and methods of disposal post-experimentation (euthanasia).

The work on the experiment listed in Aim 2 and 3 will be initiated and performed only upon review and approval of scientific intent by appropriate expert body. The study will not be initiated unless approval from Veterinary Office of Bern is received in writing.

Curriculum vitae

Name: Valentin G. DJONOV

Date of birth: March 23, 1961 in Vidin, Bulgaria

Marital status: Married to Julia Djonov, 2 children

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work: Institute of Anatomy, University of Bern
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Present position: Full Anatomy Professor /Chair

Academic degree: Doctor of Medicine and Professor of Anatomy, Histology and Embryology, University of Bern

Academic Positions:

1987-1988	General Practitioner, Vidin, Bulgaria
1988-1990	Medical Assistant, Institute of Anatomy, University of Sofia, Bulgaria
1990-1991	Medical Assistant, Division Neurosurgery, University of Sofia, Bulgaria
1991-1992	Medical Assistant, Division of General Surgery, Regional Hospital, Herzogenbuchsee, Switzerland
1992-1995	Research assistant Tiefenau Laboratory, Department of Clinical Research, University of Bern. MD thesis: Supervisor: Prof. A-C Andres
1995	Medical Assistant, Clinic of Urology, Inselspital, Bern, Switzerland
1995 – 2000	Research fellow, Division of Developmental Biology, Prof. P.H. Burri, Institute of Anatomy, Bern, Switzerland
2001 - 2002	Lecturer, Institute of Anatomy, Bern, Switzerland
November 2002	Habilitation – Anatomy, Histology and Embryology
April 2003	Assistant Professor (Dozent I), Institute of Anatomy, Bern, Switzerland
March 2006	Associate Professor of Anatomy, Histology and Embryology; Institute of Anatomy, Bern, Switzerland
Febr.- April 2007	Sabbatical, Institute of Anatomy, UCSF, San Francisco, USA
	Research group Prof. Donald McDonald
September 2007	Full professor and Co-director, Institute of Anatomy, Fribourg Switzerland
August 2010	Full Professor and Chair, Institute of Anatomy, Bern Switzerland

Grants:

1. Bernese Cancer League, 1998 - 2000 CHF 150'000.- *"The impact of angiogenesis and angiogenic growth factors on radiosensitivity of tumours of the oropharynx"*. Co-applicant with Dr. D. Aebersold, Institute of Radio-Oncology, Inselspital, Bern.
2. Bernese Cancer League, 2000 - 2002 CHF 150'000.- *"Impact of Intussusceptive Angiogenesis on tumor growth and therapy"* Principal applicant with Prof. P.H. Burri,
3. Bernese Cancer League, 2002 - 2004 CHF 120'000.-
"*Intussusceptive angiogenesis in tumors: effects of ionizing irradiation, anti-angiogenic compounds and hypoxia induced factor 1 alpha*". Principal applicant with Dr. D. Gruber, Inselspital, Bern

4. Swiss National Foundation, 2002 – 2003, CHF 120'000.- *"Hypoxia and hyperoxia: effects on lung development, intussusceptive angiogenesis and vascular plasticity"* Principal applicant with Prof. P.H. Burri and Dr. S.A. Tschanz, Institute of Anatomy, Bern
5. NOVARTIS Pharma AG, Basel, Research Agreement 2003 - 2004 CHF 105'000. per year: *"Morphological evaluation of anti-angiogenic therapies in different tumor models"* Principal applicant
6. GENCELL SAS France, Research Agreement, 2004 - 2006, CHF 138'000: *"Neovascularization and growth factor expression in the lower limb assessed in patients undergoing a major amputation."* Co-applicant with Prof. Iris Baumgartner, Clinic of Angiology, Inselspital, Bern
7. Swiss National Foundation 2004 - 2007, CHF 217'000.- *"Vascular growth and remodelling by intussusception"* Principal applicant
8. Bernese Cancer League, 2004 - 2006, CHF 60'000.- *"A two-step anti-tumor therapeutic approach using a sensitizing anti-angiogenic drug in combination with a complementary anti-angiogenic agent or ionizing radiation."* Principal applicant
9. Swiss National Foundation 2005 - 2007, CHF150'000.- *"Insufficient neovascularization in patients with peripheral arterial disease: Impaired up-regulation or failed synchronization of angiogenesis inducers "* Co-applicant with Prof. Iris Baumgartner, Inselspital, Bern
10. Swiss National Foundation 2005 - 2007, CHF 196'740.- *"X-ray phase contrast imaging of biological tissue: potential of in-line holography"* Co-applicant with PD Francis R. Verdun Institute universitaire de radiophysique appliqué, Lausanne.
11. Stiftung zur Förderung der wissenschaftlichen Forschung an der Universität Bern (Nov. 2006) CHF 10'000.-
12. Swiss National Foundation 2007 - 2010, CHF 260'000.- *"Intussusceptive angiogenesis during development, tissue repair and carcinogenesis*" Principal applicant
13. Forschungsfonds of the University of Fribourg, 2009, 10´000.-: *"The new cancer treatment modality using the synchrotron microbeam radiotherapy and antiangiogenic (chemo) therapy in human glioma xenografts in mice".* University of Fribourg, Switzerland Principal applicant
14. Joint research projects (SCOPES), Swiss National Foundation (01.01. 2010 – 31.12. 2012), CHF 210'000.- *„Uroepithelial tumors in Balkan Endemic Nephropathy - specific and common molecular pathways"* Principal applicant and coordinator
15. Swiss National Foundation 2009 – 2012, CHF 350'000.- *"Molecular mechanisms of vascular maturation for therapeutic angiogenesis"* Co-applicant with Dr. Andrea Banfi, University Basel
16. Swiss National Foundation 20011 – 2014, CHF 410'000.- *"Regulatory mechanisms of intussusceptive (splitting) angiogenesis and their potential clinical implications"* Principal applicant
17. Sciex-NMS Fellowship for project 11.233, 2012-2013, CHF 100'000.- *"Notch signaling as a potential regulatory mechanism of intussusceptive (splitting) angiogenesis"*
18. Kommission für Technologie und Innovation KTI 2012 – 2014, CHF 1'250'000.- "*Dynamic High-Resolution Microangiography*" Principal applicant
19. Swiss National Foundation 2012 – 2015, CFR 427'500.- *"Cellular and molecular mechanisms of vascular maturation for therapeutic angiogenesis "* Co-applicant with Dr. Andrea Banfi, Department Biomedicine, University Basel
20. COST, 2013-2018 around 3 Mio Euro. *Innovative methods in radiotherapy and radiosurgery using synchrotron radiation.* Co-applicant

***Awards*:**

2001: Young Investigator Award of the San Salvatore Foundation, Lugano, CHF 30'000.

2001: Gian Toendury Award Swiss Society of Anatomy, Histology and Embryology, CHF 5'000.-

2003: Teacher of the Year 2003.

2006: Secundo loco: Ordinariat in der Anatomie, University of Zurich

2006: Secundo loco: Ordinariat in der Histologie, University of Zurich

2006: Award of the European Society for Artificial Organs "Article of the year" for: *Kelm JM, Djonov V, Ittner L, Fluri D, Born W, Hoerstup SP and Fussenegger M: *Design of custom-shaped vascularized tissues using microtissue spheroids as a minimal building units. Tissue Eng 12:2151-60 (2006)* * L. Kelm and V. Djonov contributed equally to this work

2007: Lady Davis Fellowship, Hebrew University, Jerusalem

LIST OF PUBLICATIONS (Last 5 years)

ORIGINAL ARTICLES

1. Dimova I, Hlushchuk R, Makanya A, Styp-Rekowska B, Ceausu A, Flueckiger S, Lang S, Semela D, Le Noble F, Chatterjee S, **Djonov V:** Inhibition of Notch signaling induces extensive intussusceptive neo-angiogenesis by recruitment of mononuclear cells. Angiogenesis [Epub ahead of print] (2013)
2. Laissue JA, Bartzsch S, Blattmann H, Bräuer-Krisch E, Bravin A, Dalléry D, **Djonov** V, Hanson AL, Hopewell JW, Kaser-Hotz B, Keyriläinen J, Laissue PP, Miura M, Serduc R, Siegbahn AE, Slatkin DN: Response of the rat spinal cord to X-ray microbeams. *Radiother Oncol.* 106(1):106-111 (2013)
3. Ceausu RA, Cimpean AM, Dimova I, Hlushchuk R, **Djonov** V, Gaje PN, Raica M: Everolimus dual effects of an area vasculosa angiogenesis and lymphangiogenesis. *In Vivo* 27(1):61-66 (2013)
4. Gianni-Barrera R, Trani M, Fontanellaz C, Heberer M, **Djonov** V, Hlushchuk R, Banfi A.:VEGF over-expression in skeletal muscle induces angiogenesis by intussusception rather than sprouting. *Angiogenesis 2013*, 16(1):123-36
5. Dill MT, Rothweiler S, **Djonov V**, Hlushchuk R, Tornillo L, Terracciano L, Meili-Butz S, Radtke F, Heim MH, Semela D.Disruption of Notch1 induces vascular remodeling, intussusceptive angiogenesis, and angiosarcomas in livers of mice. *Gastroenterology 2012; 142(4): 967-977.e2*
6. Jacobi M, Reischl N, Bergmann M, Bouaicha S, **Djonov V**, Magnussen RA. Reconstruction of the medial ptellofemoral ligament using the adductor magnus tendon: an anatomic study.*Arthroscopy 2012; 28(1):105-9*
7. Makanya AN, Koller T, Hlushchuk R, **Djonov V**.Pre-hatch lung development in the ostrich. *Resp Physiol Neurobi 2012; 180(2-3): 183-92*
8. Salm F, Cwiek P, Ghosal A, Lucia Buccarello A, Largey F, Wotzkow C, Höland K, Styp-Rekowska B, **Djonov** V, Zlobec I, Bodmer N, Gross N, Westermann F, Schäfer SC, Arcaro A: RNA interference screening identifies a novel role for autocrine fibroblast growth factor signaling in neuroblastoma chemoresistance. *Oncogene* 10.1038/onc.2012.416 (2012)
9. Marcer N, Bergmann M, Klie A, Moor B, **Djonov V**.An anatomical investigation of the cervicothoracic ganglion. *Clin Anat 2012; 25(4): 444-51*
10. Moor BK, Kohut G, Bouaicha S, Grabherr S, Gautier E, Bergmann M, Marcer N, **Djonov V**.A pedicled bone graft from the acromion: an anatomical investigation regarding surgical feasibility. *J Shoulder Elbow Surg 2012; 21(5): 604-11*
11. Wnuk M, Hlushchuk R, Janot M, Tuffin G, Martiny-Baron G, Holzer P, Imbach-Weese P, **Djonov V**, Huynh-Do U:Podocyte EphB4 signaling helps recovery from glomerular injury.*Kidney Int.* 81(12):1212-25 (2012)
12. Makanya AN, Koller T, Hlushchuk R, **Djonov V**.Pre-hatch lung development in the ostrich. *Respir Physiol Neurobiol 180:183-92* (2012)
13. Makanya AN, Hlushchuk R, **Djonov V**.The pulmonary blood-gas barrier in the avian embryo: inauguration, development and refinement. *Respir Physiol Neurobiol 178:30-38* (2011)
14. Hlushchuk R, Ehrbar M, Reichmuth P, Heinimann N, Escher R, Baum R, Lienemann P, Makanya A, Keshet E, **Djonov V**. Decrease in VEGF expression induces intussusceptive vascular pruning. *Arterioscler Thromb Vasc Biol 31:2836-44.* (2011)
15. May D, **Djonov V**, Zamir G, Bala M, Safadi R, Sklair-Levy M, Keshet E.A transgenic model for conditional induction and rescue of portal hypertension reveals a role of VEGF-mediated regulation of sinusoidal fenestrations. *PLoS One. 2011; 6(7):e21478.* (2011)
16. Wnuk M, Hlushchuk R, Tuffin G, Huynh-Do U, **Djonov V**. Glomerular repair by intussusceptive angiogenesis in Thy1.1 nephritis – controversial effects of VEGF. *Am J Path 178:1899-912* (2011)
17. Sabatasso S, Laissue JA, Hlushchuk R , Graber W, Bravin A, Bräuer-Krisch E, Corde Tehei S, Blattmann H, Gruber G and **Djonov V.** Vascular toxicity of microbeam irradiation depends on the stage of capillary maturation. *Int J Radiat Oncol Biol Phys. 80(5):1522-32* (2011)
18. Piguet AC, Saar B, Hlushchuk R, St-Pierre MV, McSheehy PM, Radojevic V, Afthinos M, Terracciano L, **Djonov V**, Dufour JF. Everolimus augments the effects of sorafenib in a syngeneic orthotopic model of hepatocellular carcinoma. *Mol Cancer Ther.10:1007-17* (2011)
19. B. Moor, G. Kohut, S. Grabherr, S. Bouaicha, M. Bergmann, E. Gautier, **V. Djonov** A pedicled bone graft from the acromion: an anatomical investigation regarding surgical feasibility. *Int J Dev Biol. 55(4-5):343-4* (2011)
20. Andrew N. Makanya, Yosif El-Darawish, Boniface M. Kavoi and **Valentin Djonov:** Spatial and Functional Relationships Between Air Conduits and Blood Capillaries in the Pulmonary Gas Exchange Tissue of Adult and Developing Chickens. *Microsc Res Tech Feb;74(2):159-69* (2011)

21. * Boxler S, **Djonov V**, Kessler TM, Hlushchuk R, Bachmann LM, Held U, Markwalder R, Thalmann GN. Matrix Metalloproteinases and Angiogenic Factors: Predictors of Survival after Radical Prostatectomy for Clinically Organ-Confined Prostate Cancer? *AJP. 177(5):2216-24* (2010)
    * Boxler S and Djonov V contributed equally to this work
22. Baum O, Suter F, Gerber B, Tschanz SA, Buergy R, Blank F, Hlushchuk R, **Djonov V.**VEGF-A promotes intussusceptive angiogenesis in the developing chicken chorioallantoic membrane. *Microcirculation 17 (6): 445 – 57* (2010)
23. Templin C, Meyer M, Müller MF, **Djonov V**, Hlushchuk R, Dimova I, Flueckiger S, Kronen P, Sidler M, Klein K, Nicolls F, Ghadri JR, Weber K, Paunovic D, Corti R, Hoerstrup SP, Lüscher TF, Landmesser U. Coronary optical frequency domain imaging (OFDI) for in vivo evaluation of stent healing: comparison with light and electron microscopy. *Eur Hear J. 14: 1792-801* (2010).
24. Buschmann I, Pries A, Styp-Rekowska B, Hillmeister P, Loufrani L, Henrion D, Shi Y, Duelsner A, Hoefer I, Gatzke N, Wang H, Lehmann K, Ulm L, Ritter Z, Hauff P, Hlushchuk R, **Djonov V**, van Veen T, le Noble F: Pulsatile shear and Gja5 modulate arterial identity and remodeling events during flow-driven arteriogenesis. *Development;137(13):2187-96.* (2010)
25. Kemmer C, Gitzinger M, Daoud-El Baba M, **Djonov V**, Stelling J, Fussenegger M. Self-sufficient control of urate homeostasis in mice by a synthetic circuit. *Nat Biotechnol. 28(4):355-60* (2010)
26. Müller M, Beck IM, Gadesmann J, Karschuk N, Paschen A, Proksch E, **Djonov V**, Reiss K, Sedlacek R. MMP19 is upregulated during melanoma progression and increases invasion of melanoma cells. *Mod Pathol. 23(4):511-21.* (2010)
27. Ribatti D, Crivellato E, Nico B, Guidolin D, Gassmann M, **Djonov V.** Mast cells and macrophages in duodenal mucosa of mice overexpressing erythropoietin. *J Anat. 215(5):548-54* (2009)
28. Haldimann M, Custer D, Munarini N, Stirnimann C, Zürcher G, Rohrbach V, **Djonov V**, Ziemiecki A, Andres AC. Deregulated ephrin-B2 expression in the mammary gland interferes with the development of both the glandular epithelium and vasculature and promotes metastasis formation. *Int J Oncol. 35:525-36* (2009)
29. Makanya AN, **Djonov V**: Parabronchial angioarchitecture in developing and adult chickens *J Appl Physiol* 1959-69 (2009)
30. Escher R, Cung T, Stutz M, Haeberli A, **Djonov V**, Berchtold P, Hlushchuk R.:Antiaggregatory and proangiogenic effects of a novel recombinant human dual specificity anti-integrin antibody. *J Thromb Haemost 7:460-469* (2008)
31. Hlushchuk R, Riesterer O, Baum O, Wood J, Gruber G, Pruschy M, **Djonov V**: Vascular normalization by intussuceptive angiogenesis after anti-angiogenic therapy and ionizing radiation. *Am J Pathol 173:1173-85* (2008)
32. Makanya AN, Djonov V: Development and spatial organization of the air conduits in the lung of the domestic fowl, Gallus gallus variant domesticus. *Microsc Res Tech 71:689-702* (2008)
33. Sanchez-Bustamante CD, Kelm JM, Egermann M, **Djonov V**, Fussenegger M: Ectopic expression of delta FBJ murine osteosarcoma viral oncogene homolog B mediates transdifferentiation of adipose-like spheroids Into osteo-like microtissues. *Tissue Eng Part A 14:1377-94* (2008)
34. Grabherr S, Hess A, Karolczak M, Thali MJ, Kalender WA, Dirnhofer R**, Djonov V**: Angiofil®-mediated visualization of the vascular system by microcomputed tomography: a feasibility study. *Microsc Res Tech 71:551-6* (2008)
35. May D, Gilon D, Djonov V, Ivin A, Lazarus A, Rosenberge C Keshet E: A conditional transgenic system for induction and rescue chronic myocardial hibernation provides insights into genomic program of hibernation and reversible heart remodelling. *PNAS 105:282-7* (2008)
36. Grabherr S, Gygax E, Sollberger B, Ross S, Oesterhelweg L, Bolliger S, Christe A, **Djonov V**, Thali MJ, Dirnhofer R: Two-step postmortem angiography with a modified heart-lung machine: preliminary results. *Am J Roentgenol 190:345-51*(2008)

REVIEW ARTICLES AND HANDBOOK CONTRIBUTIONS

1. Makanya A, Anagnostopoulou A, **Djonov** V: Development and remodeling of the vertebrate blood-gas barrier.*Biomed Res Int.* 101597. doi: 10.1155 (2013)
2. De Spiegelaere W, Casteleyn C.Van den Broeck W, Plendl J,Bahramsoltani M, Simoens P, **Djonov V**, Cornillie P: Intussusceptive Angiogenesis: A Biologically Relevant Form of Angiogenesis. *J Vasc Res 49 :390 -404* (2012)
3. Ribatti D, **Djonov V**: Intussusceptive microvascular growth in tumors. *Cancer Lett. 316:126-31* (2012)

4. Styp-Rekowska B, Hlushchuk R, Pries AR, **Djonov V.** Intussusceptive angiogenesis: pillars against the blood flow. *Acta Physiol (Oxf). 202:213-23* (2011) *(invited article)*
5. Hlushchuk R, Makanya AN, **Djonov V**: Escape mechanisms after antiangiogenic treatment, or why the tumors are growing again? *Int J Dev Biol.;*55:563-7 (2011) (invited article)
6. Andres AC, **Djonov V:** The Mammary Gland Vasculature Revisited. *J Mammary Gland Biol Neoplasia. 15:319-28* (2010) *(invited article)*
7. Makanya A, Hlushchuk R and **Djonov V:** Intussusceptive angiogenesis and its role in vascular morphogenesis, patterning and remodelling. *Angiogenesis 12:113-23* (2009) *(invited article)*

Five most important publications:

1. Dimova I, Hlushchuk R, Makanya A, Styp-Rekowska B, Ceausu A, Flueckiger S, Lang S, Semela D, Le Noble F, Chatterjee S, **Djonov V:** Inhibition of Notch signaling induces extensive intussusceptive neo-angiogenesis by recruitment of mononuclear cells. Angiogenesis [Epub ahead of print] (2013)
2. Hlushchuk R, Ehrbar M, Reichmuth P, Heinimann N, Escher R, Baum R, Lienemann P, Makanya A, Keshet E, **Djonov V**. Decrease in VEGF expression induces intussusceptive vascular pruning. *Arterioscler Thromb Vasc Biol 31:2836-44.* (2011)
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4. Hlushchuk R, Riesterer O, Baum O, Wood J, Gruber G, Pruschy M, **Djonov V**: Vascular normalization by intussuceptive angiogenesis after anti-angiogenic therapy and ionizing radiation. *Am J Pathol 173:1173-85* (2008)
5. **Djonov V**, Schmid M, Tschanz SA, and Burri PH: Intussusceptive angiogenesis: its role in embryonic vascular network formation. *Circ Res 86: 286-292 (2000)*

Vladislav B. Volarevic

Curriculum Vitae

BORN	September 9, 1979, in Nis, Serbia
EDUCATION	Primary school ,,Saint Sava" Nis 1986-1994
	High school ,,Stevan Sremac" Nis 1994-1998
	M.D. School of Medicine University of Nis 1998-2006
	PhD studies: Immunology, School of Medicine University of Kragujevac 2006-2011
	PhD thesis entitled: ,,The role of IL-33/ST2 signalling pathway and galectin 3 in experimental model of fulminant hepatitis" defended in 2011
	Residency: Immunology, Faculty of Medical Sciences University of Kragujevac 2010-2013
POSITION	Ass. Professor Department of Microbiology and Immunology
	Faculty of Medical Sciences University of Kragujevac 2012-
RESEARCH AND TEACHING EXPERIENCE	Institute Curie, Paris, France (laboratory led by prof. Sebastian Amigorena) June-July 2009
	University of California, San Francisco, School of Medicine
	(laboratory led by prof. Abbul Abbas), August 2008
	Advanced Immunology Course, organized by AAI (American Association of Immunologists), Center for Immunology University of Minnesota Medical School. Minneapolis, August 2009
PROJECTS	1. FP7 project entitled: ,,Center for preclinical testing of active substances-CPCTAS" funded by EU 2008-2011 (position: researcher),
	2. Project ON175069 entitled: ,,Molecular determinants of innate immunity in autoimmunity and carcinogenesis" 2011-till now, funded by Serbian Ministry of science (position: researcher),
	3. Project ON175103 entitled ,, Developing infrastructure for priority research fields: Institute for Stem Cell Biology and Regenerative Medicine" 2011-till now, funded by Serbian Ministry of science (position: researcher),
	4. Project JP 02/09 entitled ,,Immunomodulation of chronic inflammatory diseases" 2009-2011, funded by School of Medicine University of Kragujevac (position: project leader),
	5. Project JP 11/10 entitled: ,,The role of T1/ST2 receptor in Concanavalin A induced hepatitis" 2010-2011, funded by School of Medicine University of Kragujevac (position: principle investigator),
PROFESSIONAL ORGANIZATION	Member of European Society of Immunologists Member of Serbian Society of Immunologist Member of Serbian Society of Medical Doctors

PUBLICATION LIST

1. **Volarevic V**, Milovanovic M, Ljujic B, Pejnovic N, Arsenijevic N, Nilsson U, Leffler H, Lukic ML. Galectin-3 Deficiency Prevents Concanavalin A- Induced Hepatitis in Mice. **Hepatology. 2012 Jun;55(6):1954-64.**
2. **Volarevic V**, Mitrovic M, Milovanovic M, Zelen I, Nikolic I, Mitrovic S, Pejnovic N, Arsenijevic N, Lukic ML. Protective Role of IL-33/ST2 Axis in Con A-Induced Hepatitis. **Journal of Hepatology 2012 Jan;56(1):26-33.**
3. **Volarevic V**, Arsenijevic N, Lukic ML, Stojkovic M. Mesenchymal Stem Cell Treatment of Complications of Diabetes Mellitus. **Stem Cells. 2011 January; 29(1): 5–10.**
4. **Volarevic V,** Ljujic B, Stojkovic P, Lukic A, Arsenijevic N, Stojkovic M. Human stem cell research and regenerative medicine--present and future. **Br Med Bull. 2011;99:155-68.**
5. **Volarevic V,** Erceg S, Bhattacharya S, Stojkovic P, Horner P, Stojkovic M. Stem cell based therapy for spinal cord injury. **Cell Transplantation 2013;22(8):1309-23.**
6. **Volarevic V**, Al-Qahtani A, Arsenijevic N, Pajovic S, Lukic ML. Interleukin-1 receptor antagonist (IL-1Ra) and IL-1Ra producing mesenchymal stem cells as modulators of diabetogenesis**. Autoimmunity. 2010 Jun;43(4):255-63.**
7. **Volarevic V,** Milovanovic M, Djekovic A, Petrovic B, Arsenijevic N, BugarcicZD.The cytotoxic effects of some selected gold(III) complexes on 4T1 cells and their role in the prevention of breast tumor growth in BALB/c mice. **J BUON.** 2010 Oct-Dec;15(4):768-73.
8. **Volarevic V**, Vujic JM, Milovanovic M, Kanjevac T, Volarevic A, Trifunovic SR, Arsenijevic N. Cytotoxic effects of palladium (II) and platinum (II) complexes with O,O'-dialkyl esters of (S,S)-ethylenediamine-N,N'-di-2-(4-methyl) pentanoic acid on human colon cancer cell lines. **J BUON**. 2013 Jan-Mar;18(1):131-7.
9. Milovanovic M, **Volarevic V**, Ljujic B, Radosavljevic G, Jovanovic I, Arsenijevic N, Lukic ML. Deletion of IL-33R (ST2) Abrogates Resistance to EAE in BALB/C Mice by Enhancing Polarization of APC to Inflammatory Phenotype. **PLoS One. 2012;7(9):e45225. doi: 10.1371/journal.pone.0045225**.
10. Milovanovic M, **Volarevic V**, Radosavljevic G, Jovanovic I, Pejnovic N, Arsenijevic N, Lukic ML. IL-33/ST2 axis in inflammation and immunopathology. **Immunol Res. 2012 Apr;52(1-2):89-99.**
11. Radosavljevic G, **Volarevic V**, Jovanovic I, Milovanovic M, Pejnovic N, Arsenijevic N, Hsu DK, Lukic ML. The roles of Galectin-3 in autoimmunity and tumor progression. **Immunol Res. 2012 Apr;52(1-2):100-10.**
12. Volarevic A, Ljujic B, **Volarevic V**, Milovanovic M, Kanjevac T, Lukic A, Arsenijevic N. A new semiquantitative method for evaluation of metastasis progression. **J BUON. 2012 Jul-Sep;17(3):585-90**.
13. Ljujic B, Milovanovic M, **Volarevic V**, Murray B, Bugarski D, Przyborski S, Arsenijevic N, Lukic ML, Stojkovic M. Human mesenchymal stem cells creating an immunosuppressive environment and promote breast cancer in mice. Sci Rep. 2013 Jul 29;3:2298. doi: 10.1038/srep02298.
14. Vujić JM, Kaluđerović GN, Milovanović M, Zmejkovski BB, **Volarević V**, Živić D, Đurđeviće P, Arsenijević N, Trifunović SR. Stereospecific ligands and their complexes. Part VII. Synthesis, characterization and in vitro antitumoral

activity of platinum(II) complexes with O,O'-dialkyl esters of (S,S)-ethylenediamine-N,N'-di-2-(4-methyl)pentanoic acid. **Eur J Med Chem. 2011 Sep;46(9):4559-65**

15. Vujić JM, Cvijović M, Kaluderović GN, Milovanović M, Zmejkovski BB, **Volarević V,** Arsenijević N, Sabo TJ, Trifunović SR. Palladium(II) complexes with R(2)edda derived ligands. Part IV. O,O'-dialkyl esters of (S,S)-ethylenediamine-N,N'-di-2-(4-methyl)-pentanoic acid dihydrochloride and their palladium(II) complexes: synthesis, characterization and in vitro antitumoral activity against chronic lymphocytic leukemia (CLL) cells. **Eur J Med Chem. 2010 Sep;45(9):3601-6.**
16. Milovanović M, Djeković A, **Volarević V**, Petrović B, Arsenijević N, Bugarcić ZD. Ligand substitution reactions and cytotoxic properties of [Au(L)Cl2](+) and [AuCl2(DMSO)2]+ complexes (L=ethylenediamine and S-methyl-l-cysteine). **J Inorg Biochem. 2010 Sep;104(9):944-9.**
17. Kanjevac T, Milovanovic M, **Volarevic V**, Lukic ML, Arsenijevic N, Markovic D, Zdravkovic N, Tesic Z and Lukic A. Cytotoxic effects of glass ionomer cements on human dental pulp stem cells correlate with fluoride release. **Med Chem. 2012 Jan;8(1):40-5.**
18. Arsenijevic M, Milovanovic M**, Volarevic V,** Djekovic A, Kanjevac T, Arsenijevic N, Djukic S and Bugarcic ZD. Cytotoxicity of gold (III) complexes on A549 human lung epithelial cell line. **Med Chem. 2012 Jan;8(1):2-8.**
19. Vujic J, Kaludjerovic G, Zmejkovski B, Milovanovic M, **Volarevic V**, Arsenijevic N, Stanojkovic T, Trifunovic S. Stereospecific ligands and their complexes. Part X: Synthesis, characterization and in vitro antitumoral activity of platinum(IV) complexes with O,O'-dialkyl-(S,S)-ethylenediamine-N,N'-di-2-(4-methyl)pentanoate ligands**. Inorganica Chimica Acta 2012; 390: 123–128.**
20. Arsenijevic M, Milovanovic M**, Volarevic V**, Canovic D, Arsenijevic N, Soldatovic T, Jovanovic S, Bugarcic Z. Cytotoxic properties of platinum(IV) and dinuclear platinum(II) complexes and their ligand substitution reactions with guanosine-5'-monophosphate. **Transition Met Chem (2012) 37:481–488. DOI 10.1007/s11243-012-9613-4**
21. Arsenijevic S, Vukcevic-Globarevic G, **Volarevic V**, Macuzic I, Todorovic P, Tanaskovic I, Mijailovic M, Raicevic S and Jeremic B. Continuous controllable balloon dilation: a novel approach for cervix dilation. **Trials 2012; 13:196. doi:10.1186/1745-6215-13-196**
22. Vukcevic G, **Volarevic V**, Raicevic S, Tanaskovic I, MilicicB, Vulovic T and Arsenijevic S. A novel semi-quantitative method for measuring tissue bleeding. Histology and histopathology, *in press*

CENTER FOR BIOENGINEERING
FACULTY OF ENGINEERING
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GENERAL INFORMATION

First and last name	Nenad Filipovic
Year and place of birth	1970, Kragujevac
Position	Professor
e-mail/web site	fica@kg.ac.rs
Telephone	+381-34-334-379
Education-scientific / education –art field	Technical science
University, faculty, organizational unit	University of Kragujevac/ Faculty of Mechanical Engineering
Field and closer speciality	Mechanical Engineering, Bioengineering

EDUCATION – DIPLOMAS

BACHELOR STUDIES	
Year	1994
Place	Kragujevac
Institution	Faculty of Mechanical Engineering
Headline of BSC	Automatically mesh generation for finite element method
Field	Computer calculations
PHD THESIS	
Year	1999
Place	Kragujevac
Institution	Faculty of Mechanical Engineering
Title of PHD thesis	Numerical solution of coupled problems: deformable solid and fluid flow
Field	Computer calculations

TECHNICAL BIOGRAPHY – POSITIONS

Year	**Position**
Research Assistant	1994
Assistant Professor	2000
Associate Professor	2005
Full Professor	2010

PROFESSIONAL BIOGRAPHY - IMPROVEMENTS
(training in the country and abroad, study courses, a visiting professor)

Year and duration	Scholarships - study abroad:
2001, 2 month	University of Vienna, Austria
2003-2009	Harvard University, USA

AWARDS AND RECOGNITIONS

Year	**Name of award/recognition**
2003	Young Scientist Award on the Second MIT Conference on Computational Fluid & Solid Mechanics, (Ed. K.J. Bathe), Boston, USA.

Ongoing Research Support

III41007 Serbian national project 01/01/11-12/31/14
Applied of biomedical engineering in the pre-clinical and clinical practice
Role: PI

FP7- ICT IP-224297 Parodi (PI) 09/01/08-08/31/13
ARTreat: Multi-level patient-specific artery and atherogenesis model for outcome prediction, decision support treatment, and virtual hand-on training.
Role: Co-Investigator, Scientific coordinator, Leader WP3

The University of Texas Health Science Center at Houston Filipovic (PI) 08/01/08-07/31/10
Division of Nanomedicine, USA
Modeling of Blood Microcirculation, Margination and Endocytosis of Particles
Role: PI

FP7 Project-NMP-2007-LARGE-1 Hack (PI) 09/01/08-08/31/13
Multifunctional materials for future vehicles, MUST
Role: Co-Investigator, coordinator for modeling

FP7 Project- 211338 Vasilis (PI) 03/01/07-02/28/10
SEE-GRID-SCI – SEE-GRID eInfrastructure for regional eScience
Role: Co-Investigator, coordinator for University of Serbia

TR12007 Serbian national project 01/01/08-12/31/10
Software and hardware development and application in the clinical practice
Role: PI

Completed Research Support

NIH/NHLBI BRP-R01 HL070542 (PI: Tsuda) 09/15/03-08/31/08
Bioengineering Research Partnership
Particles in the Developing Lung: Bioengineering Approach
To develop a combination of bioengineering tools, including computational fluid mechanics, the development of particle nanotechnology, and physiological approaches in animal models, to be utilized in a comprehensive study on particle deposition, retention, and clearance pathways in the developing lung.
Role: Co-Investigator, coordinator for modeling

NIH R01 AR48776-01A1 (Mijailovich) 9/1/03-4/30/08
Bioengineering Analysis of Muscle Mechanics and Metabolism
To investigate contractile mechanics of skeletal, cardiac and smooth muscle by engineering analysis.
Role: Co-Investigator, coordinator for modeling

MULTIMOD FP6 (Viceconti) 9/1/03-8/31/05
International Europian project for software development of clinical information system for orthopedic clinic in Bologna
Role: Co-Investigator, coordinator for modeling

PUBLICATION LIST (LAST 5 YEARS)

1	Nenad **Filipovic**, Zhongzhao Teng, Milos Radovic, Igor Saveljic, Dimitris Fotiadis and Oberdan Parodi, Computer simulation of three dimensional plaque formation and progression in the carotid artery, Medical & Biological Engineering & Computing, DOI: 10.1007/s11517-012-1031-4, (2013)
2	Parodi O., Exarchos T., Marraccini P., Vozzi F., Milosevic Z., Nikolic D., Sakellarios A., Siogkas P., Fotiadis D.I., **Filipovic** N., Patient-specific prediction of coronary plaque growth from CTA angiography: a multiscale model for plaque formation and progression, IEEE Transaction on Information Technology in Biomedicine, Vol. 16(5), pp. 952-965, (2012)
3	**Filipovic** N., Isailovic V., Djukic T., Ferrari M., Kojic M., Multiscale Modeling of Circular and Elliptical Particles in Laminar Shear Flow, *IEEE transactions on biomedical engineering*, Vol. 59(1), pp. 50-53.DOI: 10.1109/TBME.2011.2166264, (2012)
4	**Filipovic** N., Rosic M., Tanaskovic I., Milosevic Z., Nikolic D., Zdravkovic N., Peulic A., Fotiadis D., Parodi O., ARTreat project: Three-dimensional Numerical Simulation of Plaque Formation and Development in the Arteries, IEEE Trans Inf Technol Biomed, Vol. 16(2), pp. 272-278, (2012)
5	Dimkic M., Rankovic V., **Filipovic N.**, Stojanovic B., Isailovic V., Pusic M., Investigation of the M., Modeling of radial well lateral screens using 1D finite elements, Journal of Hydroinformatics, IWA Publishing.DOI:10.2166/hydro.2012.008, (2012)
6	Oberdan Parodi, Themis Exarchos, Paolo Marraccini, Federico Vozzi, Zarko Milosevic, Dalibor Nikolic, Antonis Sakellarios, Panagiotis Siogkas, Dimitris I. Fotiadis, **Nenad Filipovic**, Patient-specific prediction of coronary plaque growth from CTA angiography: a multiscale model for plaque formation and progression, *IEEE Transactions on Information Technology and Applications in Biomedicine (in press)*
7	Siogkas, P., Sakellarios, A., Exarchos, T. P., Athanasiou, L., Karvounis, E., Stefanou, K., Fotiou, E., Fotiadis, D. I.; Naka, K. K.; Michalis, L. K.; **Filipovic**, N.; Parodi, O.; Multiscale - Patient-Specific Artery and Atherogenesis Models, IEEE Trans Biomed Eng. 2011 Dec; 58(12):3464-8.
8	Z. Bosnić, P. Vračar, M. Radović, G. Devedžić, N. **Filipović**, and Igor Kononenko, Mining Data from Hemodynamic Simulations for Generating Prediction and Explanation Models, Transactions on Information Technology in Biomedicine, in press, DOI 10.1109/TITB.2011.2164546.
9	**Filipovic N**, Isailovic V, Djukic T, Ferrari M, Kojic M.,Multi-scale modeling of circular and elliptical particles in laminar shear flow, IEEE Trans Biomed Eng. PMID: 21878403, (2011)
10	**Filipovic N**, Rosic M, Tanaskovic I, Milosevic Z, Nikolic D, Zdravkovic N, Peulic A. Fotiadis D, Parodi O, ARTreat project: Three-dimensional Numerical Simulation of Plaque Formation and Development in the Arteries, IEEE Trans Inf Technol Biomed. PMID: 21937352 (2011)
11	Markovic Zoran S, Dimitric-Markovic Jasmina M, Milenkovic Dejan, **Filipovic N**, Mechanistic study of the structure-activity relationship for the free radical scavenging activity of baicalein, JOURNAL OF MOLECULAR MODELING, vol. 17 br. 10, str. 2575-2584, (2011)
12	Dimkic M, Pusic M, Vidovic D, Isailovic V, Majkic B **Filipovic N**, Numerical Model Assessment of Radial-Well Aging, *JOURNAL OF COMPUTING IN CIVIL ENGINEERING*, vol. 25 1, 43-49, (2011)
13	Markovic Z, Dimitric-Markovic J, Milenkovic D, **Filipovic N**, Structural and electronic features of baicalein and its radicals, *MONATSHEFTE FUR CHEMIE*, Vol. 142, 2, 145-152, (2011)
14	Lee G, **Filipovic N**, Lin M, Gibney B Simpson D, Konerding M, Tsuda A, Mentzer S, Intravascular Pillars and Pruning in the Extraembryonic Vessels of Chick Embryos *DEVELOPMENTAL DYNAMICS*,vol. 240 br. 6, str. 1335-1343, (2011)
15	Dimitric-Markovic M, Markovic Z. Brdaric T. **Filipovic N**. Comparative spectroscopic and mechanistic study of chelation properties of fisetin with iron in aqueous buffered solutions. Implications on in vitro antioxidant activity, *DALTON TRANSACTIONS*, vol. 40, 17, 4560-4571, (2011).
16	**Filipovic N**, Milasinovic D, Zdravkovic N, Böckler D, von Tengg-Kobligk H., Impact of aortic repair based on flow field computer simulation within the thoracic aorta, *Computer Methods and Programs in Biomedicine*, 101(3):243-52 (2011)
17	**Filipovic N**. and H. Schima, Numerical simulation of the flow field within the aortic arch during cardiac assist, *Artifical Organs*, 35, 4, 73–83, (2011)
18	**Filipovic N.**, A. Peulic, N. Zdrakovic, V. Grbovic-Markovic and A. Jurisic-Skevin, Transient Finite Element Modeling of Functional Electrical Stimulation, *General Physiology and Biophysics*, 30(1):59-65. (2011)
19	**Filipovic N**., D. Milasinovic, N. Jagic, V. Miloradovic H. Hetterich, J.Rieber, Numerical simulation of the flow field and mass transport pattern within the coronary artery, *Computer Methods in Biomechanics and Biomedical Engineering*, DOI: 10.1080/10255842.2010.482526, 14(4):379-88, (2011)
20	Kojic N., A. Huang, E. Chung, M.Ivanovic, **N.Filipovic**, M. Kojic, and D. Tschumperlin, A 3-D model

	of ligand transport in a deforming extracellular space, *Biophysical Journal*, Dec 1;99(11):3517-25.(2010).
21	**Filipovic N.**, M. Ivanovic, D. Krstajic and M.Kojic, Hemodynamic Flow Modeling through an Abdominal Aorta Aneurysm Using Data Mining Tools, *IEEE - Transactions on Information Technology in Biomedicine*, Mar;15(2):189-94 (2011)
22	**Filipovic, N.**, M. Kojic, M. Ferrari, Dissipative Particle Dynamics Simulation of Circular and Elliptical Particles Motion in 2D Laminar Shear Flow, *Microfluidics and Nanofluidics*, 10, 5, 1127-1134, 2010.
23	Lee G., **Filipovic, N.**, et al, Blood flow shapes intravascular pillar geometry in the chick chorioallantoic membrane, *Journal of Angiogenesis Research*, 2:11, 2010
24	**Filipovic, N.**, Vulovic, R., Peulic, A., Radakovic, R., Kosanic, Dj. and Ristic., B. Noninvasive determination of knee cartilage deformation during jumping, *Journal of Sports Science and Medicine*, 8, 584-590, 2009.
25	**Filipovic, N.**, A. Cvetkovic, V. Isailovic, Z. Matovic, M. Rosic and M. Kojic, Computer simulation of flow and mixing at the duodenal stump after gastric resection, *World Journal of Gastroentrology*, 15 (16), 1990-1998, 2009
26	**Filipovic, N.**, AkiraTsuda, Grace S. Lee, Lino F. Miele, Miao Lin, Moritz A., Konerding, and Steven J. Mentzer, Computational Flow Dynamics in a Geometric Model of Intussusceptive Angiogenesis, *Microvascular Research* Dec;78(3):286-93, 2009.
27	Tsuda, A., **Filipovic, N.**, Haberthür, D., Dickie, R., Matsui, Y., Stampanoni, M. and Schittny, J.C., Finite element 3D reconstruction of the pulmonary acinus imaged by synchrotron X-ray tomography, *J Appl Physiol* 105: 964-976, 2008
28	**Filipovic, N.**, M. Ivanovic, M. Kojic, A comparative numerical study between dissipative particle dynamics (DPD) and smooth particle dynamics (SPH) when applied to simple unsteady flows, *Microfluidics and Nanofluidics*, 1613-4982, 2008.
29	D. Milašinović, M. Ivanovic, H. Tengg-Kobligk, D. Böckler, **N. Filipović**, Software Tools for Generating CFD Simulation Models of Blood Flow from CT Images, and for Postprocessing, *Journal of the Serbian Society for Computational Mechanics*, 2, 2, 51-58, 2008.
30	O. Miljkovic, M. Ivanovic, **N. Filipovic**, M. Kojic, AI Models of the Hemodynamic Simulation, *Journal of the Serbian Society for Computational Mechanics*, 2, 2, 59-72, 2008
31	Kojic, M., **Filipovic, N**., Stojanovic B., Kojic N., **Computer modeling in bioengineering: Theoretical Background, Examples and Software**, John Wiley and Sons, Chichester, England, 2008
32	**Filipovic, N**., Haber, S., Kojic, M., Tsuda, A., Dissipative particle dynamics simulation of flow generated by two rotating concentric cylinders: II. Lateral dissipative and random forces, *J. Phys. D: Appl. Phys.* 41 035504 (6pp) doi:10.1088/0022-3727/41/3/035504, 2008
33	**Filipovic, N.**, Kojic, M., Tsuda, A., Modeling thrombosis using dissipative particle dynamics method, Phil Trans Royal, A 366(1879), 2008
34	Kojic, M., **Filipovic, N.,** Tsuda, A., A mesoscopic bridging scale method for fluids and coupling dissipative particle dynamics with continuum finite element method, *Comput. Methods Appl. Mech. Engrg*. 197, 821–833, 2008.
35	**Filipovic, N**., Ravnic, D.J. Kojic, M., Mentzer, S.J., Haber, S. Tsuda, A., Interactions of Blood Cell Constituents: Experimental investigation and Computational Modeling by Discrete Particle Dynamics Algorithm, *Microvascular Research*, 75, 279-284, 2008.
36	Rosic, M., Pantovic, S. Rankovic, V. Obradovic, Z. **Filipovic, N.** Kojic, M., Evaluation of dynamic response and biomechanical properties of isolated blood vessels, *J. Biochem. Biophys. Methods*, Vol 70, 6, 966-972, 2008.
37	Milasinovic D, Ivanovic M., **Filipovic N**, Kojic M, Software tools for automatic generation of finite element mesh and application of biomechanical calculation in medicine, HEMIJSKA INDUSTRIJA, vol. 62 no. 3, pp 177-180, (2008).

Ivanka I. Dimova

Curriculum Vitae

BORN	December 14, 1973, in Dupnitza, Bulgaria
EDUCATION	High school of life sciences, Dupnitsa, Bulgaria 1987-1991
	MD in Medical University of Sofia, Bulgaria 1991-1997
	PhD studies: Genetics, Medical University of Sofia, Bulgaria 2001-2006
	PhD thesis entitled: ,,Genetic characteristics of different types of ovarian tumors" defended in 2006
	Residency: Medical genetics, Medical Faculty of Medical University of Sofia 2001-2013
POSITION	Assoc. Professor (Docent) in Department of Medical genetics
	Medical Faculty of Medical University of Sofia 2012-
RESEARCH AND TEACHING EXPERIENCE	"Novel technologies in diagnostics of hereditary diseases and predispositions" – Sofia, Bulgaria 2002
	"VYSIS FISH Application" – Wiesbaden, Germany 2003
	International training course of Institut Pasteur, Paris "Methodes Moleculaires de Detection et de Typage des Microorganismes"– Sofia, Bulgaria 2003
	"Novel strategies in diagnostics and therapy of cancer", University I Montpellier, France 2004-2005
	Training course on the Transgenomic WAVE System and Navigator Software, Sofia, Bulgaria 2005
	Training course "Clinical ArrayCGH Workshop", Department of Pathology, University of Cambridge, UK 2007
	"Basic principles of lentiviral transfection", University of Berne, Switzerland 2008
	Training course "Animal models in cell therapy and tissue engineering", University of Geneva, Switzerland 2010
	"Standartization and improvement of pre-analytical tools and procedures for in vitro diagnostics", SPIDIA-RNA Program 2010, Italy
	Classical and Quantitative real-time PCR analysis, Byosystems Ltd., Sofia, 2010
	Next Generation Sequencing (NGS), MiSeq – Illumina, Sofia 2012
	SNP microarray analysis by iScan, Illumina, Medical University Istanbul, Turkey, 2012
PROJECTS	"Novel strategies in cancer diagnosis and therapy", EUROGENDIS Training Site, Montpellier, France, Laboratory "Genotypes et phenotypes tumoraux", Contract N° QLGA-CT-2000-60005 – main applicant
	«Study of copy number changes of oncogenes PRAD1 and ZNF217 in ovarian tumors with different clinico-pathological characteristics», Contract № 2/2004, MSC, Medical University Sofia - researcher

"Bulgarian Center of Excellence for complex diseases", Ministry of Education and Science, Contract № 05/01.08.200 - researcher

„Bulgarian Consortium for Structural genomics and In Silico Drug design » (Project No DRI-5/2006, MES) - researcher

"Expression levels of hTERT and EGFR mRNA as biomarkers for early detection of non small cell lung cancer", MES, Contract No ВУ-Л-316/07 - researcher

"High throughput analysis by DNA microarrays for idiopathic assospermia in sterile men", Contract №39 /2007, MSC, Medical University Sofia - researcher

"Whole genome screening for microstructural unbalanced chromosomal aberrations in hemihyperplasia and Proteus syndrome" Contract No5, 2007, MSC, Medical University Sofia - researcher

„Expression profiling for factors of immunity in patients with bronchial asthma" MSC, Contract No -45 -Д/2008 – researcher

„Microstructural unbalanced genome alterations in patients with idiopathic mental retardation" MSC, Contract No -28 -Д/2008 - researcher

„Combined expression analysis of genes, connected to neoangiogenesis, in non-small cell lung cancer" Contract No 15-Д/2008, Medical University Sofia - researcher

„High resolution analysis for prognostic markers in high grade glial tumors of central nervous system", Contract No 25-Д/2008, Medical University Sofia - researcher

"Proteome screening in serum of patients with schizophrenia in different stages and types of therapy" , MES, Contract No 02.12/10.02.2009 - researcher

„High resolution genome screening for unbalanced genome alterations in children with congenital malformations" Contract №46/ 2009, Medical University Sofia - researcher

"Molecular karyotyping in patients with congenital malformations", MES, ДТК 02/76-21.12.2009 - researcher

"Uroepithelial tumors associated with Balkan Endemic Nephropathy – specific and common molecular pathways", Contract IZ73Z0_127949/1, SCOPES 2009, SNSF, Switzerland – researcher

"Whole genome methylation analysis in BEN patients using DNA microarrays", Ministry of Education and Science of Bulgaria, Contract DMU03/35/12.12.2011, fund of 44 000 BGL – project leader

"Notch signaling as a potential modulator of intussusceptive (splitting) angiogenesis", Scientific exchange between Switzerland and New member states of EU, CRUS, Switzerland, fund of 95 200 CHF – awarded fellow

PROFESSIONAL ORGANIZATION

Member of European Society of Human genetics
Member of Bulgarian Society of Human genetics
Member of Swiss Society of Anatomy, Histology and Embryology

PUBLICATION LIST

Original publications (last 5 years)

1. **Dimova I**., Hlushchuk R., Makanya A., Styp-Rekowska B., Ceausu A., Flueckiger S., Lang S., Semela D., Le Noble F., Djonov V. Inhibition of Notch signalling induces extensive intussusceptive neo-angiogenesis by recruitment of mononuclear cells. Angiogenesis 2013 Oct;16(4):921-37.
2. Stoynev N., **Dimova I**., Rukova B., Hadjidekova S., Nikolova D., Toncheva D., Tankova T. Gene expression in peripheral blood of subjects with hypertension and subjects with type 2 diabetes. J Cardiovasc Med (Hagerstown). 2013 Jan 18.
3. Ceausu RA, Cimpean AM, **Dimova I**, Hlushchuk R, Djonov V, Gaje PN, Raica M. Everolimus dual effects of an area vasculosa angiogenesis and lymphangiogenesis. In Vivo. 2013 Jan-Feb;27(1):61-6.
4. Damyanova V., **Dimova I**., Savov A., Nesheva D., Hadjidekova S., Rukova B., Jivkova R., Nikolova V., Vatev I., Toncheva D. Comprehensive genomic study in patients with idiopathic azoospermia and oligoasthenoteratozoospermia. Biotechnology & Biotechnological Equipment (27) 2013 No 1: 3529-3533.
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Scientific Committee
SCOPES Program
Swiss National Foundation
Wildhainweg 3
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Berne, 18 Sept. 2013

Dear Members of the Scientific Committee,

We would like to submit the enclosed grant proposal entitled "**ROLE OF BLOOD FLOW AND SDF-1/CXCR4–INDUCED RECRUITMENT OF MONONUCLEAR CELLS IN INTUSSUSCEPTIVE ANGIOGENESIS**" for evaluation by the Scientific Committee of the Swiss National Foundation.
This project resulted as a natural follow up of past and recent scientific collaborations between the participants. The research groups of Dr. I Dimova from Bulgaria, Dr. V. Volarevic and Dr. N Filipovic from Serbia are tightly linked and between the groups a long term partnership existed. Dr. Dimova was temporary a part of my research team in the last years which resulted in fruitful collaboration and excellent scientific output.
Dr. Filipovic is one of leader in computational simulation in the field of intussusceptive angiogenesis and made an important contribution in the field during his time at Harvard medical school.
Dr. Volarevic is an excellent young researcher with scientific topic in the field of stem cells and their impact in tumorigenesis and angiogenesis.

I would like to mention the fact, that the co-applicants have outstanding expertize in their fields and their complementary skills are very important for the successful implementation of the project.

Recognition by the Swiss National Foundation will greatly encourage our research endeavours, and its financial support will be invaluable in furthering our knowledge and understanding of cellular and molecular mechanisms of angiogenesis.

Yours sincerely,

V. Djonov

Valentin Djonov MD

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