KDUIRC
INTERNATIONAL RESEARCH CONFERENCE

15TH INTERNATIONAL RESEARCH CONFERENCE

Economic Revival, National Security, and Sustainability through Advancement of Science, Technology, and Innovation

29TH - 30TH SEPTEMBER 2022

MEDICINE

PROCEEDINGS

GENERAL SIR JOHN KOTELAWALA DEFENCE UNIVERSITY

15TH INTERNATIONAL RESEARCH CONFERENCE

ECONOMIC REVIVAL, NATIONAL SECURITY, AND SUSTAINABILITY THROUGH ADVANCEMENT OF SCIENCE, TECHNOLOGY, AND INNOVATION

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Welcome Address

Major General Milinda Peiris RWP RSP VSV USP ndc psc MPhil (Ind) PGDM
Vice Chancellor, General Sir John Kotelawala Defence University

Chief Guest, Secretary - Ministry of Defence, General Kamal Gunaratne (Retd), Keynote Speaker, Hon. Prof. Subramanian Swamy, Your Excellencies in the Diplomatic Corps, Chief of Defence Staff, Gen Shavendra Silva, Commander of the Army, Lt Gen Vikum Liyanage, Commander of the Navy, Vice Admiral Nishantha Ulugetenne, Eminent plenary speakers representing our friendly nations, Vice Chancellors of Other Universities, Former Commandants of KDA, Former Chancellors and Vice Chancellors of KDU, Rectors of KDU Campuses and Deputy Vice Chancellors, Deans of Faculties and Centre Directors, Senior Military Officers and Police officers, Academics, Administrative Staff, Students, All distinguished guests including those who connected with us in the cyberspace, Ladies and gentlemen, Good Morning to you all! I am deeply honoured to make the welcome address at this inauguration of the 15th International Research Conference (IRC) of General Sir John Kotelawala Defence University. To begin with, I warmly welcome our chief guest this morning, Gen Kamal Gunaratne (Retd), Secretary to the Ministry of Defence for gracing this important occasion. We owe you a great deal of respect for the whole-hearted support extended for the progression of this university at all times. Also, may I have the distinct honour of welcoming our keynote speaker, the esteemed and renowned personality, Hon Prof Subramanian Swamy from neighbouring India.

Hon Sir, we are extremely grateful to you for accepting our invitation and honouring us with your gracious presence to deliver the keynote address of this two-day international research conference. I am sure that your eminent presence adds great value to the event, and we are looking forward to listening to your words of wisdom, which will surely set the most appropriate tone for this scholarly event.

I also welcome the Chief of Defence Staff, Gen Shavendra Silva, Commander of the Army, Commander of the Navy and all other members of our Board of Management. Let me also warmly welcome the members of the Diplomatic Corps representing our friendly nations, Vice Chancellors and Senior Academics from other universities, Former Commandants of KDA, Former Chancellors & Vice Chancellors of KDU, Other officials of Ministry of Defence, Academics, Senior Military Officers, Plenary speakers, Scholars presenting papers in this two-day conference, and all other distinguished invitees and students joining this event physically as well as on cyberspace. As the Vice Chancellor of KDU, I admire your valuable presence at this occasion.

Reflecting on KDU IRCs held last year and the year before, we held them under the most trying circumstances of the grave pandemic. They really tested our resilience and

defiance against challenges to the very core. Along with the IRCs, we determinedly continued with all academic and other activities of the university with much vigor, and the results are evident in our achievements.

Ladies and gentlemen, today, we are glad that KDU has firmly established its foot print as a unique higher educational model in the world, which even its critics would not be able to disagree with. The best evidence is its steady growth in its popularity as an Higher Education Institute in Asia, as well as the quality of its output, which are evident in the Times Higher Education Impact Ranking, 2022 table, where KDU is ranked 2nd in Sri Lanka for Quality of Education and 4th in the overall ranking in the country and in the 801-1000 range globally. A more recent indicator of our growth is evident in the world ranking of Law Schools, where the KDU faculty of law took a leap in the world ranking from the 498th place in 2021 to the 83rd place in 2022, from the 189th place to the 25th place in Asia, and from the 5th place to the 2nd place in Sri Lanka.

Ladies and gentlemen, today, we hold the 15th consecutive IRC at a time when we, Sri Lankans are in a grave need to pull up our socks as a nation to face the seemingly unsurmountable economic crisis we are in. And we as a university are determined to give our utmost best for the nation at this crucial juncture.We believe that the role of the universities and the intellectual community of the nation is of paramount importance for the resurrection of our economy, and that of the nation's defence university is even more significant as it deals with the national security perspective which is inseparably linked with the economic crisis and with a possible recovery from the same.

Serious research in defence and security studies needs to go hand in hand with rigorous research in all other fields. This, we believe, is an essential prerequisite for a quick and sustainable recovery from the crisis. So, we carefully selected the overarching theme, *"Economic Revival, National Security, and Sustainability through Advancement of Science, Technology, and Innovation"* for this year's conference, and its scope encompasses a wide range of significant research possibilities to engage in.

Our aim in selecting this theme entails a holistic vision of the complexities of economic and national security perspectives which demand comprehensive inter- and multidisciplinary approaches to resolve contemporary issues. The expectation is to carry forward the research outcomes to the attention of those in authority to consider implementation to resolve related issues. I do not intend to talk any further on this aspect as I am sure our keynote speaker would elaborate on the conference theme and its significance. Ladies and gentlemen, having commenced in the year 2008 in a humble way, the KDU IRC gained gradual momentum as a trustworthy forum for the country's scholarly community to showcase their multi-disciplinary research outcomes. And what is noteworthy is the ever growing increase in the number of research papers submitted for the conference, and more so is

the increasingly higher quality of the papers presented at the conference.

Therefore, KDU enjoys the humble pride of its leading role in strengthening the research culture in the country that is more and more inclined towards product based or problemsol ving outcomes in relevant fields, which I believe is the need of the hour. Also the involvement of internationally collaborative research is on the increase. Anyone who visits the KDU IRC Proceedings would note the evolutionary path of the progression in research in the country spearheaded by KDU – You could see the increasingly high numbers of researchers representing almost all the universities, other Higher Education Institutes and research institutes of the country as well as those from renowned universities, Higher Education Institutes and research institutes in the world. So, we are proud of our role in establishing local and international research and scholarly networks that would further enhance creation of new knowledge in diverse disciplines and dissemination of the same.

Ladies and gentlemen, the organizers of this year's research conference too have been doing their utmost best to maintain and upgrade the quality of the annual research conference despite challenges, especially in the face of financial constraints which compelled them to significantly cut down on peripheral expenses.

The circumstances have compelled them to rely on our own resources as much as possible, which I believe is a blessing in disguise in the crisis situation to convert challenges into opportunities. I appreciate their effort and the support extended from all quarters to make the KDU International Research Conference a resounding success in terms of achieving its objectives. So, let me conclude by once again welcoming our chief guest, the erudite keynote speaker, and all the other distinguished invitees. I convey my congratulations to all researchers who will be presenting their research during the couple of days.

I also request those whose papers were not selected through the double blind reviewing process not to get disheartened because you had competed with many for a placement in the conference. Finally, let me express my heartfelt thanks to the Chairman of the Conference Organizing Team, Dr. Kalpa Samarakoon, Secretary, Dr. Pandula Athawuda Arachchi and the other members of the team for the tireless hours, days and weeks you spent to see the success of this important event.

May the KDU IRC be a haven for establishing scholarly links at national and international levels, which would pave the way for fruitful research, academic and even industrial collaborations for the betterment of our nation, its security and its social, economic and political stability that would in turn pave the way for the creation of a self-sufficient nation in the not so long future. Let us optimistically believe in ourselves and in our potentials to reach that target sooner than later.

Thank you.

Chief Guest Speech

General Kamal Gunaratne (Retd) WWV RWP RSP USP ndc psc MPhil
Secretary - Ministry of Defence, Sri Lanka

Hon. Prof. Subramanian Swamy, Keynote speaker of the 15th International Research Conference 2022 of General Sir John Kotelawala Defence University, Your Excellencies in the Diplomatic Corps, Chief of Defence Staff, Commander of the Army, Commander of the Navy, Chief of the Staff of Sri Lanka Air force, Vice Chancellors of Other Universities, Vice Chancellor of KDU, Eminent speakers from friendly foreign nations, former commandants of KDA, former Chancellors and Vice Chancellors of KDU, Rector of KDU Metropolitan Campus, Rector of KDU Southern Campus and Deputy Vice Chancellors, Deans of Faculties and Directors, Senior Military Officers and Police officers, Distinguished guests, Ladies, and Gentlemen's. Good morning to all of you.

I consider it as a great pleasure and privilege to be present here today as the chief guest of the inauguration ceremony of General Sir John Kotelawala Defence University's International Research Conference, which is taking place for its 15th consecutive time.

Without a doubt it provides as opportunity for academics, professionals, researchers and practitioners from all around the world to share their research findings and expertise addressing mutual challenges in their fields. Further it provides an opportunity for a wide interaction and networking with national and international scholars in respective fields which in turn proved beneficial for the participants to broaden their horizons of knowledge through intellectual discussions most importantly despite the global pandemic situation and the reason economic, social and political setbacks in effect it is truly inspiring to see that the KDU is continuation the conduct of this conference with renewed spirit and commitment

Therefore, ladies and gentlemen at this moment I would like to encompass
My sincere appreciation to the Vice Chancellor and the conference organizers for the invitation extended for me to be the chief guest to the most significant academic events of this University. In this context of promoting an excellent academic culture generation of knowledge and subsequent applications of it led to innovations and novel technologies that are crucial for the advancement of humanity, well-being, and sustainability. The knowledge is generated by scientific research and at this backdrop, it is delightful to see that the theme of this year's conference reads economic revival, National Security, and Sustainability through the advancement of Science, Technology, and Innovations, which is a well-timed theme reflecting directions that we should pursue as a country irrespective of the boundaries of time and era.
Further, at this moment, ladies and gentlemen, I will be failing in my duty if I do not acknowledge the distinction of a brilliant keynote address conducted by the former Minister of Commerce Law and the Justice

Republic of India, Honorable Professor Subramanian Swamy. Sir, we as Sri Lankans truly appreciate the accept acceptance of our invitation extended to attend and maintain throughout the past in continuation of the display of your friendliness towards Sri Lanka. The ideas that would be shared by you in this eminent forum today will indeed bring a sparkling light to the discussions to be conducted during this conference that will become highly fruitful with your intellectual input.

All the foreign and the local participants including the senior officers od tri-forces and police would be immensely benefited by the inputs that would be given by you to broaden the Horizon of their knowledge.

Moving on the the focus of the conference I must emphasized that with the effects of globalization in effect the growing international independencies affecting the Sri Lankan National security as well as reasons concerns raised by economic and political implications. There is a recognized need for assessment of the potential to national security, that may emerge during the thrive towards revival of national economy and sustainability.

As per my belief given the importance of certain sectors to the effective functioning of the Sri Lankan society the said need for a deeper conceptual understanding of the threats that may impact the implied economic revival and sustainability in all aspects focusing on technological scientific and innovative faces would be comprehensively discussed with in the earnest gathering of intellectuals during these two days.

A strategic standpoint keeping the past and also most recent lessons learned

In mind a newfound leadership of the present government, Sri Lanka should call for national determination where all sectors of Sri Lankan society including civil organizations, security institutions, political entities and business associations come together to discuss fundamental issues such as national identity, national reconciliation, transitional justice, governance structure, economic revival and many more.

This is a fundamental step towards building consensus and religious legitimizing state institutions and private organizations in the country towards a common goal. Not only would such an effort-based process serve as the foundation for a national pact addressing the country's issues, pointing out how it would concurrently compel every group in society to work towards state building and the sustainability of a secure country due consideration to scientific and technological innovations.

Furthermore, giving high priority to providing solutions to the country's most freezing matters of concern to improve the world's image of Sri Lankans society the Sri Lankan government must take every step necessary to recover high-priority initiatives in the fields of the economy, institution-building, and political reform.

Whilst giving true meaning to the said initiatives in order to address emerging challenges promoting more research and development becomes a task of topmost priority bestowed upon all of us who are present here today.

Fortunately, as a secretary Defence and the Chairman of the KDU Board of Management, I

feel tremendously proud and content to state that KDU is at the forefront of researching the development and security related problems holistically.

In this context, one of the unique aspects of KDU IRC in comparison to s plethora of symposia that we witness in the country and beyond its borders remains to be its firm commitment to defence and strategic aspects of the contemporary world with emphasis on local and regional trends.

In that this conference continues to pioneer in upholding the notion that security is a prerequisite for the viability of achievements in all other areas in which mankind relies on in order to facilitate such outcomes it maintains a seamless association of defence and security with other core areas such as Sciences, Medicine, Engineering, Build environment and Spatial Sciences, Technology, Management, and Huminites. We are fundamental knowledge images. To be honest, I personally acknowledge this pragmatic philosophy as a remarkable achievement of KDU and thereby of the country as a whole. Resulting in interactions and dialogue across apparently distinct disciplines will certainly usher increasing exchanges and collaborations among experts in diverse areas, therefore, I am well certain that all faculties of Sir John Kotelawala Defence University with their interest and commitment to knowledge in diverse academic disciplines and outside researchers' inputs would contribute immensely to this year's research conference theme.

The knowledge that you are giving to another and sharing during this conference would be an immense benefit not only to the academic community but to the entire humankind to make their lives better.

In conclusion, ladies and gentlemen, at the current context we are on the average of striving to accomplish serenity and excellence in an economic revival, national security, and sustainability through unexploited frontiers of technological innovations as a nation. Therefore, conferences of this nature are instrumental in clearing our fond of mind for the betterment of establishing solutions, therefore, let me express my sincere appreciation to the Vice Chancellor and organizers of the 15th KDU IRC 2022 for inviting to this occasion as the chief guest and giving me an opportunity to speak to you. Let me appreciate all the efforts and congratulate all of you for working your way towards a timely and appropriate theme. Finally, I wish all the participants all the very best in their research endeavors and the KDU research conference for 2022 to be successful in every way.

Keynote Speech

Hon Prof Subramanian Swamy
Former Minister of Commerce, Law & justice, India

Hon. Professor Subramanian Swamy, former Cabinet Minister of India made insightful remarks in the keynote address and initiated his speech by extending his gratitude towards Vice chancellor Major General Milinda Peiris for the invitation bestowed on him and went on to acknowledge the presence of the chief guest, Secretary to Ministry of Defence, General Kamal Gunaratne stating, how the Indians themselves couldn't put an end to a major terrorist problem in the region. Professor Swamy recollected how Sri Lanka has never been defeated throughout history, exempting a few setbacks. Furthermore, Professor Swamy remarked how the 21st century isn't going to distinguish between large nations and small nations, as it's a new era with innovations. Speaking from his experience as a trained economist, Professor Subramanian Swamy recalled how all economic development took place when the share of innovation calculated within the GDP rounded up to at least 55%, indicating the development of the USA, Europe and China as examples. He explicating further, mentioned that the growth rate of GDP would be dependent upon the extent to which one innovates. Professor Swamy also recognized the role that could be assumed by the universities in the development of the concept of innovation.

Professor Swamy, elaborated on the inception of the definition of – National security relating to its historical context. He expressed that for most of the 20th century national security had been a matter of military power, and explicated with the dawn of the 21st century, non-state actors posed most of the challenges to national security as opposed to conventional military warfare. Moreover, professor Swamy emphasized that long-term unsustainable practices make the state more vulnerable to internal and more resilient to external threats. Professor Swamy pointed out the "economic factor "as the primary reason behind Sri Lanka's recent upheaval. Furthermore, he scrutinized the removal of democratically elected people from office, which in turn would disallow them to complete their full term, which he recognized as a blow to the country's national security.

Professor Swamy detailed important aspects that need to be regarded in policy formulation; clearly defined structure of objectives, the order of priorities, strategy to achieve them, and resource mobilization. He also stated that no country should be too dependent on one country, and pointed out how Sri Lanka owes a single country, a staggering 52% in internal and external debt. He further resonated that the world has moved from the notion of "development" to "sustainable development", "sustainable economic development and sustainable national security" during the course of the last thirty years of the 20th century. Professor Swamy asserted that the most stable system of governance is democracy. Furthermore, he perceived economic security, political security, energy security, homeland security, and new

technology and innovations to be primary elements that constitute sustainable national security. Honourable professor Subramanian Swamy concluded his speech by stating that the sustainable national security of a country is the ability to provide comprehensive protection and holistic defence of citizenry and climate change, other issues of globalization, terrorism and many more.

Vote of Thanks

Dr Kalpa W Samarakoon

Conference Chair, 15th International Research Conference,
General Sir John Kotelawala Defence University

The Chief Guest, General Kamal Gunarathne, Secretary to the Ministry of Defence, The keynote speaker, Hon Prof Subramanian Swamy, Chief of the Defence Staff, Commander of the SL Army, Commander of the SL Navy, The Representative of the Commander of the SL Air force, The Vice Chancellor of KDU, The Rector KDU Southern Campus, The Rector KDU Metropolitan Campus, The Deputy Vice-Chancellor (Defence & Administration), The Deputy Vice-Chancellor (Academic), Deans of Faculties, Directors, Senior Professors, Senior Officers of tri-officers, and Police, Distinguished invitees, Colleagues, ladies, and gentlemen. Good morning!

Sri Lankans have been suffering an economic slowdown in the post covid era, in particular, with a social and economic crisis, food insecurity, and inequitable provision of health and education, due to its over-reliance on traditional exports, tourism, and constant geopolitical battles. In this context, KDU has been successful in organizing its 15th consecutive International Research Conference. We, strategically analyzed the role of academia of the country to collectively come together and facilitate the transfer of knowledge, skills, and solutions using science, technology, and innovation.

The IRC theme selection for 2022, aims to provide a multi-professional platform to all the scholars based in Sri Lanka and overseas to bring in their innovative research ideas to fulfil this national responsibility thrust upon us, to revive the nation's economy, to achieve sustainable economic growth coupled with an environment of justice and enhanced security for all. This year's conference attracted more than six hundred and ninety paper submissions in 11 sessions the highest-ever submissions since the inception of IRC. This indicates the amount of novel knowledge generated in our country. This year is the conference's inaugural technology and criminal justice sessions.

With deep appreciation and gratitude, I would like to express my heartiest thanks to General Kamal Gunaratne, the secretary to the Ministry of Defence who is our Chief Guest today at KDU-IRC 2022. Sir, your gracious presence in this occasion despite other commitments is truly appreciated and encouraging, and it has certainly added glamour and value to this important event on the KDU calendar. The same goes with Hon. Prof. Subramanian Swamy. He is a renowned academic and has been a distinguished politician in India and even beyond. Sir, I greatly appreciate your willingness to be our keynote speaker. It is truly an honour, privilege, and inspiration to witness your presence among the KDU community today.

I would like to take this opportunity to express my heartfelt gratitude and deep appreciation to the Vice Chancellor of General Sir John Kotelawala Defence

University, Maj. General Milinda Peiris, with your leadership, guidance, and timely decisions, prevailed throughout the event organization. The event would not be bound to be a success without your active input, particularly under the current difficult context. Thank you indeed Sir.

I will be failing in my duties if I didn't acknowledge the crucial involvement of KDU Deputy Vice-Chancellor (Defence and Administration), Brigadier W. Chandrasiri. He in fact steered KDU-IRC 2022 organization effort providing correct and pragmatic directions successfully even when the team was at difficult crossroads. I would also like to thank the Deputy Vice-Chancellor academic and all faculty Deans and Directors, who held the responsibilities for organizing and conducting forthcoming academic sessions.

Ladies and Gentlemen, as I said before, It has been a seemingly overwhelming challenge to organize, coordinate and conduct a research conference of this magnitude at this time.

I must appreciate the support of our sponsors. Platinum Sponsors, together with banking giants namely, Bank of Ceylon, People's Bank, and special sponsors, Gamma interpharm and George Stuart Health.

Let me take this opportunity to thank generously, conference secretary, Dr Pandula Athaudaarachchi, Senior lecturer and consultant interventional cardiologist, and the tremendous work done by the three co-secretaries, Dr. Gihani Jayaweera, Lt Col Lasitha Amarasekara and Ms. Sandali Goonathilaka, who stood alongside me ever since work has been commenced in mid of 2022 with exceptional commitment. I also thank all the session coordinators who supported tirelessly around the clock from the moment. I am certainly indeted to them for the success of KDU-IRC 2022.

I deeply appreciate all the presidents of the committees, and committee members, faculty committees, Office of Vice-chancellor, Office of DVC, officers of Bursar, Officers of the registrar, Adjutant, co-admin who held and executed the roles and responsibilities over the IRC. A special thank goes to the media and communication team led by the Director of IT, Publishing, printing and editorial committees.

I take this opportunity to thank all authors who shared their valuable research works at KDU-IRC. I thank both internal and external reviewers who perused and evaluated the submissions. Please be assured that your expertise shown and valuable time spent in critical reviewing is duly appreciated.

An event of this dimension cannot happen overnight. The wheels start rolling months in advance, it requires meticulous planning and execution and an eye for details. I cannot thank everyone enough for the involvement they have shown, So please bear with me if I would not have named all the supporters.

I expect that participants of the two-day conference that commenced just now will have an occasion that broadens their horizons of own know-how and improve networking in a refreshing environment which all of us at KDU has attempted to facilitate.

I wish you the very best at the conference.

Thank you very much!

MEDICINE

PLENARY SESSION

Health Financing for Sustainable Development: Current Challenges and Future Policy Plans

Dr. Asela Gunawardena

Director General, Ministry of Health, Sri Lanka

The health system of Sri Lanka, as a model, is a good example of the best universal health coverage. Healthcare system in Sri Lanka is considered to be a highly successful low cost model. Further, it's highlighted by community focused health care system by

- Free at the point of delivery
- Sound primary healthcare policy
- Widespread healthcare services.

With those highlights, Sri Lankan model has done well in the millennium hitting the developed good health indices.

It is important that Health Financing strategies to be considered within the broader development strategies. The fundamental sources of financing health care, yet this are an area that has been overlooked. Therefore it is necessary to pay greater attention to the economics of health policy. As we all understand rising health expenditure, enhanced productivity, this welfare gains for all. Thus, proper health financing policies are very important as it is the main determinant of the quality of health service.

Toward ensuring Universal Health Coverage, domestic resource utilization is vital. Yet the moot point is: whether the current economic conditions permit the governments of many countries around the world to enhance the budgeting allocation for health.

Economic growth experienced by many countries around the world, especially before the advent of COVID-19 pandemic, push many countries to the category of middle income countries. This has increased the availability of domestic funds for the health sector.

Yet these funds should be utilized efficiently and it is important that these funds are channelled towards priority populations and services to ensure equitable access to quality health services and to provide financial protection for all.

Advent of the COVID-19 pandemic, while dragging the entire world economy into recession as never before, has brought unprecedented attention to the issues of health system sustainability.

In spite of many limitations and constraints, Sri Lanka has performed much better in containing the spread of the deadly virus.

However, the economic recession that resulted from the COVID-19 pandemic endangered Universal Health Coverage endangering equity. Therefore, proper and strong health financing policies with adequate concessions towards the needy lower income population is a must to overcome the current economic constraints.

Health, Human Capital, and the Economy

Dr. Ajay Tandon

Lead Economist, World Bank, Washington DC, USA

Human capital – the knowledge, skills, and health that people accumulate throughout their lives, which enables them to realize their potential as productive members of society – is critical to the development of individuals and communities and to countries' economic growth. In addition to the massive economic fallout, COVID-19 has had a negative impact on human capital, including and especially on health and education, reversing years of gains made across many countries. To the extent COVID-19 presents an opportunity, it is one for removing any doubts that health and the economy are inextricably linked and that these links are bi-directional. Furthermore, the way health systems are organized and financed impacts both health and economic outcomes. Hence, in defining and measuring universal health coverage (UHC), these linkages are made explicit. In thinking and dialoging on health, especially from the perspective of public finance, it is important to underscore the importance of health not just for improving health but also for improving the effectiveness of investments in other sectors such as education, for enhancing labor productivity, generating employment and improving income-generating potential, and for accelerating poverty reduction efforts. Fiscal policies for reducing harmful consumption of tobacco, alcohol, sugar, and polluting fuels can help improve health but also can help governments expand revenues. Hence, health should be viewed as an investment "not as consumption" with wide ranging economic returns.

Importance of Public Health Resilience in the New Normal

Dr. Alaka Singh

WHO Country Representative, World Health Organization, Sri Lanka

Sri Lanka has consistently achieved remarkable health outcomes for its income group. This sustained success is rooted in the primary health care (PHC) orientated health systems approach that pre-dates Alma-Ata with public investment in public health. Pre-COVID-19, efforts were focused on tackling the increasing public health challenge from NCDs and integrating the area into Sri Lanka's primary health care. NCDs have underlined the multiple and complex influences on health, beyond the health sector alone, and which required a multi-disciplinary, multi-sectoral and whole-of-society approach to secure the 'best buys', notable prevention of NCDs. COVID-19 has changed global public health forever. Sri Lanka's success with vaccinations and managing hospital care demonstrated the capacity of the country's PHC system to adapt and innovate to emergency public health needs - the essential attributes of resilience. There are also important lessons learned from the pandemic for public health. Mental health has emerged as a key NCD challenge that further underscores the significance of a multi-disciplinary and whole-of-society approach, especially engagement with communities for psycho-social support as a critical component of mental health care. Communities have, in fact, proved to be critical contributors to overall service resilience, meriting a formal role for non-government entities in public health in future. This shall require a broader

review of human resources as the key to sustainable resilience as highlighted by COVID-19, including skill mix and capacities in using the potential of technology for health. The prolonged global pandemic brought with it an unprecedented economic downturn with recovery predicted to be slow. Superimposed on this in Sri Lanka is the worst economic crisis since independence. For Sri Lanka, the new normal shall be in the context of this dual challenge. Public health has been built on the sound principles of primary health care and these need to be preserved. Further, lessons learned for resilience from the pandemic need to be examined for the systematic strengthening of primary health care going forward. WHO is supporting analytical work in each health systems area to identify options for recovery in Sri Lanka based on both technical issues as well as international experience.

Reforming for better Primary care in economic crisis situation

Dr. Susie Perera

Deputy Director General, Public Health Services, Ministry of Health, Sri Lanka

People-centered comprehensive primary care to all was the underlying strategy in the policy for health care delivery for Universal health coverage in 2018. Since then major reforms were envisaged through technical support from WHO and also financial support from ADB and the WB. Project mode approaches were adopted, with the intention of being aligned with Primary care strengthening goals stated in the policy.

Repurposing of project funds during the COVID 19 pandemic, which is to support other medicinal supplies in the economic downturn, makes Sri Lanka move further away from the envisaged reforms to establish person-centered, comprehensive and continuity of care that can be accessed at the primary care level. The Shared care cluster model for re-organizing is doable through both projects as interventions can be aligned with the cluster concept, which utilizes the existing service structure optimally. COVID-19 response in Sri Lanka was an opportunity missed to initially continue to attract patients to their closest health facility, instead patients were hospitalized at a higher-level facility and taken to distant places for isolation. As the response matured and the numbers increased, home-based care was adopted through doctors on call who were virtually present and supported by the community health staff when follow up was needed. The 960 primary care health facilities could have provided a system, linking people to their closest health facility to home. Primary care strengthening is still valid as a cost-effective health care reform in the economic crisis. It needs essential primary health care package to be redefined, rational human resource planning, competencies for family medicine, management reforms to ensure accountability, robust data management and preservation of the existing community health services, retooling to ensure proper integration at the primary care level the vertical run national programs, support of the specialists to provide rational continuity of care for patients. The presentation explores the different health systems' operational policies that must converge to support the overall policy for primary care strengthening.

TECHNICAL SESSION

Analysis of Interleukin-6 and Interleukin-8 in a Cohort of Patients with Colorectal Cancers in Sri Lanka

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Abstract*: Colorectal cancer (CRC) is one of the most prevalent cancers globally, accounting for nearly 10% of all cancers. Interleukin-6 (IL-6) and Interleukin-8 (IL-8) levels have been reported to increase in CRC patients. The studies on IL-6 and IL-8 levels of CRCs have confined to Caucasian populations and levels of these cytokines have not been extensively investigated in South Asian populations. They have the potential of using as markers but are not being used in clinical practice, yet. Therefore, the aim of this study was to investigate the serum IL-6 and IL-8 levels in a cohort of Sri Lankan patients. Blood samples from thirty five patients with CRCs and thirty five healthy volunteers were obtained after informed consent. The concentrations of IL-8 and IL-6 were measured using ELISA according to manufacturer's protocols. The mean serum concentration of IL-6 was found to be significantly higher in the CRC patients than controls ($p<0.05$). Although the mean serum concentration of IL-8 was higher in the CRC patients than controls the difference was not significant ($p>0.04$). Interestingly, the mean serum [IL-6] in colorectal cancer patients were correlated with the disease stage. The study provided preliminary evidence to use IL-6 as potential biochemical marker to be used in the diagnosis of CRCs. However, it is necessary analyze more patient samples to validate the results of this study.*

Keywords: *Colorectal cancer, Interleukin-6, Interleukin-8, Serum, Diagnosis*

1. Introduction

Colorectal cancer (CRC) is one of the most prevalent cancers globally, accounting for nearly 10% of all cancers (Sung et al, 2020). It affects over 1.93 million people globally and over 0.9 million deaths have been reported in 2020 (WHO, 2022). There has been a rise in both morbidity and mortality over the years, with predicted rise by about 80% in 2035 (Douaiher*et al*., 2017). Majority of CRCs are sporadic (70 – 80%) (Yamagishi *et al*., 2016) and the remaining are classified as familial (20-25%) (Lichtenstein P, et al 2000) and inherited (5%) (Jasperson et al 2010). Development of CRCs occurs due to alterations of the genetic composition and environmental factors (Rattray *al*., 2017). Inflammation has been identified as a key determinant in CRC pathogenesis and progression (Long *et al*., 2017).

Aberrations in the signalling pathways lead to the abnormal production of cytokines. (Klampfer L., 2011). Cytokines are known to be inflammatory mediators that determine both pro-tumorigenic and anti-tumorigenic signals within the tumour environment (Shrihari TG, 2017). Both systemic and local changes in cytokine profiles have been observed in CRCs (Akhmaltdinova *al*., 2020). Interleukins are types of cytokines, which have been identified to play a role in tumorigenesis angiogenesis, cancer cell invasion, metastasis of CRCs (Pretzsch et al., 2019). Therefore, it is important to study interleukins in CRCs as they play a role in the

development, progression and survival of patients (Park, et al, 2020).

Interleukin-6 (IL-6), a central player in CRCs, is a prototypic inflammatory cytokine (Waldner *et al*., 2012) that is overexpressed in CRC tissues (Nagasaki *al*., 2014) and is known to be involved in the development of sporadic CRCs (Waldner *et al*., 2012). It acts as a growth factor for human CRC cells (Sun et al., 2020). This inflammatory cytokine is secreted by stimulated monocytes, fibroblasts, endothelial cells, macrophages, T-cells and B-lymphocytes (Akira *et al*., 1993). Serum IL-6 levels are elevated in CRCs and correlates with large tumor size, advanced stage, occurrence of liver metastases and reduced survival (Vainer *al*., 2018). Moreover, increased blood IL-6 concentration in CRC patients is an adverse prognostic marker of survival (Shiga *et al*., 2016). DNA mismatch repair defects, angiogenesis (Tseng-Rogenski *et al*., 2015, Wang et al 2020) and accumulation of myeloid-derived suppressor cells in tumors are directly promoted by IL-6, facilitating tumour progression (Lin *et al*., 2020).

Interleukin-8 (IL-8) is an inflammatory cytokine that is mainly produced by macrophages, T cells, B cells and plays a vital role in the inflammatory response of cells (Gonzalez-Aparicio 2022). IL-8 expression is upregulated in tumour tissue of CRC patients (Rubie *et al*., 2007). *In vitro* experiments done on CRC cell lines have shown that IL-8 promotes tumour growth, cell proliferation, metastasis, angiogenesis (Rubie *et al*., 2007) and chemoresistance (Burz *al*., 2021). IL-8 influences the growth and invasion of CRC cells through various mechanisms. An increase in serum IL-8 levels correlates with high tumour grade, increased invasion into the liver, growth and progression of the tumour (Rubie *et al*., 2007), all of which accounts to poor prognosis of CRCs (Xia *et al*., 2015).

Cytokine concentrations in blood have been investigated in studies confined to Caucasian populations. Data on South Asian populations is sparse. Increasing research evidence show that they can be used as markers for CRCs but are not being used in clinical practice, yet. Therefore, it is worth to assess the use of them as biomarkers for CRCs which has not been evaluated adequately in South Asian populations.

The aim of this study was to investigate serum IL-6 and IL-8 levels in a cohort of Sri Lankan patients with benefits of developing potential non-invasive biomarkers which may be useful for the diagnosis, prognosis and efficacious therapeutic approaches for CRCs.

2. Methodology

A. Patient selection

Thirty five patients diagnosed with colorectal cancer reported to the University Hospital of General Sir John Kotelawala Defence University (UHKDU) and National Cancer Institute (NCI), Sri Lanka during the period of January 2021 to December 2021 were recruited for the study after the informed consent. This study was conducted in accordance with the Helsinki Declaration and ethical clearance was obtained from the Ethics Review Committee of the Faculty of Medicine, General Sir John Kotelawala Defence University. Permission was obtained from oncologists and oncological surgeons to recruit their patients. Patients and volunteers were recruited only after written informed consent. Patients who were not able to provide written informed consent, patients under 18 years of age, patients who have undergone treatment for colorectal cancer (surgery/chemotherapy/radiotherapy) and patients with other cancers, chronic infections, HIV, chronic diseases, diabetes, immune disease, cardiovascular and cerebrovascular disease were excluded. The control group consisted of thirty five healthy volunteers, with no

comorbidities and family history of malignancy, who visited the Blood Bank of UHKDU.

B. Samples

Demographic and relevant clinical data were recorded. Whole blood samples (3–5 mL) were collected in plain tubes containing no anticoagulant, and transported to the laboratory at 4 ℃. Samples were obtained at the time of diagnostic/follow up blood sampling and from the same blood donation cannulation in the control group. Serum was separated by centrifugation at 1000xg for 15 minutes in a refrigerated centrifuge and stored at -80 ^{0}C until use. Serum was analysed after histology was confirmed. The samples were stored with a code assigned to it instead of the participant's name.

C. Enzyme-linked immunosorbent assays

All serum samples were removed from –80 ^{0}C and left to thaw on ice at room temperature before analysis. Serum IL-8 (n=35) and IL-6 (n=15) of patients and controls were analyzed using commercial ELISA kits (Elabscience). The assays were performed according to the manufacturer's instructions. All samples were tested in duplicates.

D. Statistical analysis.

T-test was used to investigate if there is a significant difference between serum IL-6 and IL-8 cytokine levels in patients and the control group. *P*-values less than 0.05 were considered to be statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 28.

3. Results & Discussion

A. Demographic characteristics

The mean age of patients recruited in this study was 64 years (41-82 years) and that of controls was 32 years (24-50 years). Majority of both patients and controls were males. Demographic and clincal data are shown in table 1. The tumors were mostly left sided (85.7%), moderately differentiated (71.4%) and were adenocarcinomas (91.4%). Most of the tumors were at stage III (51.4%), while 17.1% were advanced stage IV tumors. The mean CEA cencentration was observed to be high in majority of patients (≥5.0 ng/dL).

B. Serum cytokine concentrations

The mean concentration for IL-8 was 38.16 pg/ml (n=35) and it was higher than those of controls IL-8 = 33.67 pg/ml (n=35). The mean concentration for IL-6 was 46.31pg/ml (n=15) and for controls it was 11.15 pg/ml (n=15). There was no significant difference between the IL-8 levels of CRC patients and the control group ($p > 0.05$). Interestingly the mean serum concentration of IL-6 was found to be significantly higher in the CRC patients than controls ($p=0.04$). However, the sample number for IL-6 was 15 as there were descripancies between the duplicate values of some samples (CV>20). However, the research is ongoing to repeat the ELISA in those samples and to have more sample numbers (n=50). Similar observations have been reported in the previous studies (Shiga *et al*., 2016, Groblewska *et al*., 2008). The elevations of IL-6 have been hypothesized to be a causative factor of various cancers and to be related to prognosis (Giessen et al 2014 and Huang et al 2015).

C. Relationship of Serum cytokine concentrations with disease stage

Previous studies have reported a relationship between serum interleukin levels and disease status in CRC patients (Shiga *et al.,* 2016 and Yeh *et al.,* 2010). Chung et al. reported that tissue expression of IL-6 may represent a useful predictor of prognosis in CRC. In an attempt to identify a relationship of IL-8 and IL-6 with the

Table 1: Demographic and clinical characteristics of patients

Variables		Patients (P) (n=35)	Controls (C) (n=35)
	Age (Years)	41-82	24-50
	Mean	65 +/- 11	32 +/- 7
Gender	Male	24 (68.6%)	25 (71.5%)
	Female	11 (31.4%)	10 (28.5%)
Histology	Adenocarcinoma	32 (91.4%)	
	Singlet-ring cell carcinoma	1 (2.86%)	
	Not available	2 (5.71%)	
Grade	Well-differentiated	3 (8.6%)	
	Moderately differentiated	25 (71.4%)	
	Not available	7 (20%)	
Location	Right sided	3 (8.57%)	
	Left sided	30 (85.7%)	
	Transverse colon	2 (5.71%)	
Stage	I	1 (2.9%)	
	II	3 (8.5%)	
	III	18 (51.4%)	
	IV	6 (17.1%)	
	Not available 7	(20%)	

disease stage, it was observed that the mean serum [IL-6] in the CRC patients increase with the disease stage (Stage I: 0.16 pg/ ml; stage II: 7.01 pg/ ml; stage III: 15.8pg/ ml, and stage IV: 35.48pg/ ml). IL-8 did not show a positive relationship with the disease stage.

4. Conclusion

Although further studies are needed with higher sample numbers, data from this study provides clear preliminary evidence to use IL-6 as a potential biochemical marker for CRCs. Although previous studies have shown that IL-8 could be a potential marker for CRC, the data from the present study did not provide clear evidence to support previous findings for IL-8.

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Stem cell manipulation with plasmid-based transcription factor over-expression systems to successfully generate pre-specified cells fates in-vitro; proof of concept of in-vitro stem cell fate reprogramming using cDNA vectors

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Abstract*: The differentiation of stem cells in a controlled fashion is essential to achieve a predefined daughter cell types required for research or regenerative therapies. Transcription factors play a key role in switching cellular differentiation fate in-vivo, at initiation of neuro or glial cell fate specification phase in rodents and humans. The aim of the study was to assess if stem cell differentiation can experimentally be manipulated using expression of cDNA of regulatory homeodomain transcription factors Olig2, Nkx2,2 or Ngn2 in-vitro mirroring the in-vivo development.*

Mouse embryonic stem (ES) cells and human foetal neural stem (FNS) cells were cultured according to standard protocol. pIRES plasmid vector system with Olig2 transcription factor expression, with or without the co-transcription factor Nkx2.2 (or Ngn 2), were created using molecular biological techniques and introduced into differentiating stem cells. Using biomarkers, final cell fates were compared with one another, including a placebo version.

Both mouse embryonic and human neural precursor cells can be made to prematurely differentiate towards neuroglial fate with forced expression of Olig2 transcription factor, whereas co-expression of Olig2 and Nkx2.2 leads to premature oligodendroglial fate specification, compared to placebo. The quantitative effect of fate switching was marked with embryonic stem cell differentiation.

Forced expression of key transcription factors as illustrated, may be an attractive method to control stem cell fate modification in in-vitro, and this may successfully be used to generate rare live human cells (such as Oligodendroglia or other specialised cells) for further experiments.

Keywords: *Stem cell re-programming, fate modulation, Transcription factors*

1. Introduction

Provision of rare cells such as human cardiomyocytes, neural precursors and oligodendroglia, for in-vitro study of effects of various drugs and for studying disease models is a much sought-after goal of many research groups working in new therapeutic aspects.
Stem cells, that are thought to mediate the replacement of many cell types of the body following trauma or disease processes, have been investigated for their regenerative potential. However, achieving the exact internal

milieu required for this process is difficult to recreate in-vitro. Therefore, genetic manipulation such as selective, transcription factor modulation has been sought as an attractive method to achieve large numbers of desired cells in-vitro for experiments.

The study of the process of neuro glial specification from multipotent stem cells provides a valuable insight into the importance of transcriptional factors in regulating the cell fate decisions during the process of development. This presented an opportunity for us to investigate whether in-vitro mimicry of this process using the over-expression of key transcription factors may provide a system stem cell fate modulation.

Olig2, described initially as a transcription factor important in the specification of oligodendrocytes (Zhou et al., 2000),(Lu et al., 2000) has been since then been demonstrated to be linked to the specification of motoneurons and oligodendrocytes (Takebayashi et al., 2000),(Zhou and Anderson, 2002),(Lu et al., 2002) in the ventral spinal cord, as markers in neoplastic disease(Lu et al., 2001), in the early stages of specification of astroglia in the neural precursors in the subventricular zone(SVZ)(Marshall et al., 2005) and ependymal cells (Masahira et al., 2006).

Several groups have attempted the strategy of overexpression of olig2 to specify oligodendroglia from stem cell populations such as olfactory ensheathing cells (Zhang et al., 2005), and mouse neural stem cells (Copray et al., 2006). Human foetal neural stem cells/precursors present an attractive prospect of a precursor cell type that has a better safety profile with their lineage restriction compared with ES cells, but have sufficient immaturity to attempt directed differentiation to generate human oligodendroglia. However, these stem cells/precursors have not been investigated for their full potential for generation of oligodendroglia.

We have performed a" placebo" controlled experimental study of the role of Olig2 (with or without Nkx2.2) in the specification of glia from neural stem cells, derived from embryonic stem cells from a system devoid of morphogens and also from cultures of human foetal neural stem cells, in order to understand some of the multiple roles for Olig2 in operation in such systems. We discuss the use of the gene over-expression strategies for generating human oligodendroglia, using a plasmid vector driven, constitutionally expressed transcription factor system.

2. Materials and Methods

A. Cell Culture

Mouse ESCs were cultured as previously described and differentiated in COM (Wiles and Johansson, 1999), (Bouhon et al., 2005), (Bouhon et al., 2006). ESCs were maintained by routine feeder-free culture on 0.1% gelatine(Stem Cell Inc, Temecula) in lscove's Modified Dulbecco's Medium (IMOM)/ GlutaMax I (lnvitrogen, Carlsbad, CA, http://www.invitrogen.com) supplemented with 15% foetal calf serum (Bio Sera, Ringmer, East Sussex, U.K., http://www.biosera .com), penicillin/streptomycin, nonessential amino acids (lnvitrogen),2-mercaptoethanol (Sigma-Aldrich, St. Louis, http://www.sigmaaldrich.com), and 10 ng/ml leukaemia inhibitory factor (Chemicon, Temecula, CA, http://www.chemicon.com). ESC lines E14, and W9.5 (with feeders for maintenance, followed by feeder free passaging) were used. For terminal differentiation, enzymatically dissociated ESCs were resuspended in COM and plated at $4*10^6$ cells per 16 ml in 10-cm plastic culture dishes (Grainer Biochem). The technique for successful culture of embryonic stem cells in a morphogenic signal free environment in-vitro has been previously described by our group

(Joannides et al, 2008) and (Athauda-arachchi, 2009).

Human foetal neural stem cells were cultured as previously described (Svendsen et al., 1998). Human foetal neural tissue was acquired, governed by local ethical guidelines, from terminated foetuses between 8 and 12 weeks in gestation. Automated tissue chopping with Mcllwain tissue chopper (Mickle Engineering, UK) was used for establishment of primary cultures and passaging. The cultures were established in T75 flasks (lwaki), with Dulbecco's Modified Eagles Medium (DMEM) and Ham's F-12 medium 2:1 with GlutaMax I, 2% B27 supplement and 1x penicillin/ streptomycin (all from lnvitrogen). Growth factors were used as described previously. Cell cultures were fed every 3rd day and passage between 3 and 6 passages before terminal differentiation. For terminal differentiation, single cell suspensions were made by enzymatic dissociation of neural precursors thus cultured and plated on poly-ornithine (POrn,Sigma) and laminin-1 (Sigma) at 10 μg/ml coated coverslips at 250,000 viable cells per coverslip and cultured in Dulbecco's modified Eagle's medium/2% B-27 (lnvitrogen), 1% N2(Invitrogen). Following trophic factors were used (post nucleofection) to support terminally differentiating progeny: Glial Derived Neurotrophic Factor (GDNF) at 10ng/ml and Brain Derived Neurotrophic Factor (BDNF) and recombinant Platelet Derived Growth Factor-a (PDGF- a) at 10ng/ml (all from R&D).

Human Embryonic Kidney (HEK) cells were cultured in T25 flasks (lwaki) in DMEM and 10% foetal Calf serum and passaged as per standard procedures.

B. Construction of a plasmid vector system with constitutive Olig2/Olig-Nkx2.2 or Nkx2.2 expression

plRES plasmid vector with cytomegalovirus promoter (pCMV) was used to construct a gene expression system. The following cDNA (original cDNA clones: gift from Dr. Toru Kondo) was used to construct two types of plasmid vectors: mouse olig2 cDNA in multicloning site A alone or along with rat Nkx2.2 cDNA cloned into multicloning site B. A plasmid construct with only Nkx2.2 in multicloning site B was also constructed. Control vectors included pl RES vector with no cDNA inserts and those with enhanced green fluorescent protein (eGFP). Standard molecular biology procedures were followed and accuracy of cloned sequences and their up and downstream sites were checked by gene sequencing. Mass scale plasmid production was performed and plasmid DNA was purified using an endotoxin free preparation method (Qiagen, Endo free Maxi kit).

C. Transfection of HEK cells, mouse ES and human foetal neural stem cells

Delivery of the plasmid vectors into cultured HEK cells, mES and hFNS cells were performed by nucleofection using standard procedures recommended by the manufacturer (Amaxa, GmBH). These cell lines were transfected with a variety of programmes and presence of transgene expression confirmed by immunohistochemistry and western blotting and the most efficient transfection parameters were deduced for mES and hFNS cells. In the case of mES cells, transfected cells were selected for G418 resistance for 2 weeks and cell lines with stable transgene expression were amplified prior to differentiation. They were also checked for continued expression of pluripotency markers prior to differentiation. In the case of human foetal neural stem cells, transient transfection followed by terminal differentiation was performed without the use of G418.

D. lmmuno-cytochemistry

lmmuno-cytochemistry was performed on either free-floating whole-sphere (whole-mount) cryostat sections or plated cells as described. Primary antibodies used were the following: Anti- Oct4(1: 100, Santa Cruz) Anti-Olig2 (1:20,000; gift of Dr. David Rowitch); anti-Nkx2.2(1:50 from Developmental Studies Hybridoma Bank, Iowa City, IA, http://www.uiowa.edu/_dshbwww); anti-Human nestin (1:400 Chemicon); anti- Rat nestin (1:400; BD Pharmingen, San Diego, http://www.bdbiosciences.com/pharmingen) ; anti-Beta-Ill- tubulin (1:200; Sigma-Aldrich); anti- EGFR (1:50; Affinity Bioreagents, Golden, CO, http://www.bioreagents.com); anti-glial fibrillary acidic protein (anti-GFAP) (1:200; DAKO, Glostrup, Denmark,http://www.dako.com);Anti-O4, anti- A2B5 and anti-GalC supernatants(1:5 live staining, (Sommer and Schachner, 1981));. Secondary antibodies (lnvitrogen) were used at concentrations of 1:1,000.

Glass coverslips thus prepared were mounted in Vectafluor mounting medium (Vector Laboratories; Burlingame, CA, USA) and viewed a Leitz microscope with appropriate filters for cell identification and counting. For each coverslip, three consecutive random fields were counted using a grid, and all cells on each cryostat tissue section were counted. The number of positive cells was expressed as a mean± S.E.M. from 3 slides in each experiment. Each experiment was repeated at least 3 times

D. Western Blotting

Transfected HEK cells and mES cells after long term selection were grown to 70% confluency were used to prepare protein lysates by the following washing in PBS and suspension in RIPA buffer(PBS, SOS 1%, Triton-x100 1%, Na deoxycholate 0.5%) with Mini-complete protease inhibitor(Roche) and lysed using Lysing Matrix C Ribolyser beads and a Ribolyser. The lysate was then centrifuged at 14000 rpm for 20 minutes at 4°C and supernatant collected for freezing at -20 ^{o}C or for the use in experiments.

Protein quantification was done by copper sulphate and bicinchonic acid method (SCA assay (Pierce Kit). Lysate containing 20 ug of protein was used for 15% Sodium Dodecyl Sulphate- Polyacrylamide gel electrophoresis (SDS-PAGE) with PVDL blot transfer and detection. SDS- PAGE was performed for approximately 3 hours using constant voltage of 40V. Transfer was done at constant direct current at 300 mA for 1 hr. Primary antibodies used were olig2 (1:40,000; gift of Dr.David Rowitch); anti-Nkx2.2(1:100 from Developmental Studies Hybridoma Bank, Iowa City) and secondary antibodies used were Horseradish peroxidase conjugated secondary antibodies.

E. Co-immuno-precipitation

Co-immuno-precipitation was performed to demonstrate the presence of interaction between mouse Olig2 and rat Nkx2.2 in HEK cells using immunoprecipitation starter pack (GE Healthcare Inc), using Sepharose H beads soaked in RIPA lysis buffer to adsorb complexes saturated with Anti-Nkx2.2(DSHB 1:100) overnight and detecting the complexes with western blotting using Anti-olig2(1:40,000 gift from Dr. D. Rowitch).

F. RT-PCR analysis of gene expression

Day 8 early neurospheres derived from the mES cells with transfected plRES-Control (referred to as the Ctrl) and plRES-Olig2 (referred to as the test) plasmids, were snap frozen in -80oC or added with RNAse later (Qiagen) prior to storage in -80°C.These were then used for extraction of mRNA. This involved the use of RNAeasy RNA extraction kit (Qiagen), according to the manufacturer's instructions.

The mRNA thus obtained was assessed for quantity and purity, using a spectrophotometer (Gene Quant Pro). cDNA was synthesized using Superscript Ill (lnvitrogen) and random hexamers according to manufacturers' instructions. One microlitre each of cDNA was used to analyse for the presence of the transcripts.

3. Results

A. Accuracy of gene sequences, mRNA & Protein expression:

Independent gene sequencing was carried out (Lark Technologies, Takeley, UK) and checked for expression of Olig2 at protein level in human & mouse cells by western blotting and demonstrated that the Olig2 and Nkx2.2 expressed by this system form a physical complex by PCR, co-immunoprecipitation and immunohistochemical evidence of expression (Fig 1,2 ,3,4,5).

B. Mouse ES cells fate regulation

At the commencement of the experiment, the cellular morphology and expression of pluripotency marker Oct-4 were comparable in both the controls and Olig2 over-expressing mES lines. The only difference detected was the expression of Olig2.

Terminal differentiation of early (day8) neural precursors for 10 days demonstrates that in the presence of Olig2 over-expression, premature gliogenesis occurs when compared to the controls which generate a predominant neuronal phenotype (Fig 6). Quantification reveals that 10 days post-plating, GFAP positive cells arise (52.82% +/-1.75% n=3) with a reduction of ß -I11-tubulin positive cells (40.48%+/-2.89% n=3) in the presence of Olig2 misexpression, in sharp contrast to the controls where a predominance of ß -11I-tubulin positive cells (91.88%+/-2.01%, n=3) were present, without any cells expressing GFAP (0%, n=3) (Fig 7). The GFAP positive cells with Olig2 misexpression also co-stained for S100f3 but do not stain for nestin (figure 8). Olig2 over expression also leads to the presence of bipolar A2B5 positive, nestin negative cells at 5 days post plating (figure 8), but does not generate O4 positive cells at day 10 (0%, n=3) under these conditions.

In contrast to the above, terminal differentiation of early (day8) neural precursors for 10 days in the presence of over-expression of both olig2 and nkx2.2, O4 positive cells were also analysed (6.083%+-/0.57% n=3) (figure 11). Some of these O4 positive cells also stained for GalC (1.86%+/-0.15% (n=3)) and appeared morphologically larger and more arborised; whilst the remainder were only expressing O4 and were comparatively smaller, reminiscent of early oligodendroglia (fig 10).

In the presence of Nkx2.2 and Olig2 expression, the total number of glial cells does not differ significantly from the situation when only Olig2 is over-expressed (48.68%+/-4.34% n=3 and 52.82%+/-1.75% n =3 respectively, figure 10). Further, there is no significant difference in the beta-11I- tubulin positive cell numbers in the two situations above (39.45%+/-2.92% n=3 and 40.48%+/-2.89% n=3 respectively). This implies that presence of Nkx2.2 is immaterial to the neuronal-glial fate decision making which therefore is entirely dependent on the presence or absence of Olig2 misexpression in this system. In contrast, Nkx2.2 in the presence of Olig2 is likely to affect an oligodendroglial-astroglial fate decision after the neuronal-glial fate decision has occurred.

C. Human neural stem cells fate regulation & demonstration of specification of human oligodendroglia in-vitro

We examined the fate of human foetal neural stem cells subjected to transient over-expression of Olig2 with or without Nkx2.2(Fig 5). The transfection efficiencies for these cells

were low (27.41%+/- 1.36%) even under optimal conditions. However, this was still sufficient to specify O4 expressing cells after 14 days of terminal differentiation , when both Olig2 and Nkx2.2 were over-expressed (3.087%+/-0.49%, n=3). There were also O4 and GalC double positive arborized cells with typical human oligodendroglial morphology at day 14 (1.75%+/-0.226%, n=3), (figures 12,13). Olig2 alone was not sufficient to specify O4 positive cells, neither were the controls.

4. Discussion

A. Premature acquisition of gliogenic competency during the generation of mouse ES derived early neural precursors and human neural precursors triggered by over-expression of key transcription factors

Directed differentiation of neuronal and glial precursors needs a thorough understanding of how cell fate decisions occur during development can be studied using ES neuralisation. The radial glial specification and their transition from an early neuronal to late glial competency in the context of ES differentiation is a much-discussed phenomenon (Liour et al., 2006), (Pollard and Conti, 2007). Further, tissue derived neural precursors seem to exhibit a similar pattern in development through radial glial stages in-vivo(Gotz and Huttner, 2005; Misson et al., 1988). Transcription factors have been described to affect the progeny specified from radial glia in culture (Hack et al., 2004).

Therefore, along similar lines, we investigated whether Olig2 alone or in combination with its binding partners, can accelerate the process of acquisition of glial competency. A major practical problem in elucidating the true effect attributable to a transcription factor is the necessity in tissue culture for added morphogens, such as retinoids and sonic hedgehog, which could confound the perceived effect. Therefore, we chose to study the effect of olig2 on neural precursors developing from embryonic stem cells in an in-vitro model of neuralisation, which does not employ the use of such morphogens. We also investigated the potential of utilising such a strategy for generating human oligodendroglia.

We have demonstrated that olig2 can accelerate the process of achieving a premature glial competency. Given the similarity of sub-ventricular zone derived precursors/radial glia to ES derived neural precursors (Liour et al., 2006), our findings are keeping with the observations made by two other groups for a proposed novel role for olig2 in the process of differentiation of subventricular zonal precursors to astrocytes as well as oligodendrocytes (Marshall et al.,2005),(Masahira et al., 2006) and also indirectly supports the theory of glial restricted progenitors arising during neural development(Mayer-Proschel et al., 1997), (Rao and Mayer-Proschel, 1997). We also demonstrate that the technique of transcriptional factor control can be applied to reveal the presence of a considerable oligodendroglial potential in human foetal neural stem cells, which have a much-limited nascent potential to differentiate into many cell types, compared to ES cells. Therefore, this may be a safer and efficient method to attempt direction of differentiation to achieve human oligodendroglial cells for research.

B. Proof of concept of directed stem cell differentiation, using cDNA vectors in-vitro for diagnostic & therapeutic applications

Extrapolation of these results suggests that the co-operative action of Olig2 and Nkx2.2, which are highly conserved transcription factors in evolution, irrespective of the source of cDNA extraction, under optimal DNA transfection or transduction conditions, should be able to restore the oligodendroglial

potential in a vast majority of cultured human foetal neural stem cells as well as embryonic stem cells, mirroring normal embryonic development. Therefore, we conclude that directed differentiation of human foetal neural stem cells or embryonic stem cells, under relevant transcriptional regulation, may represent an attractive option for the generation of rare human cells such as oligodendroglia, illustrating potential for stem cell based diagnostic or therapeutic applications in many other fields such as neurology, cardiology or endocrinology.

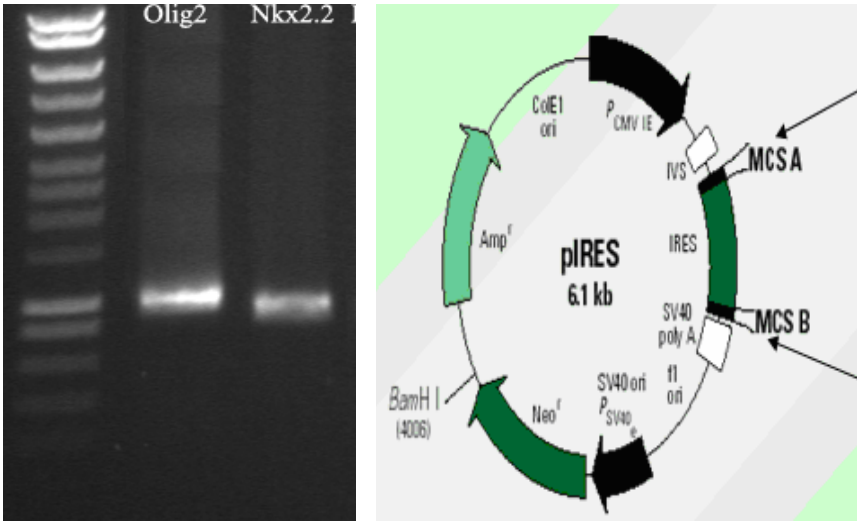


Fig 1:
Cloned PCR amplicons Olig 2 and Nkx2.2, from cDNA library, and intended multicloning sites (MCS) A or B of plamid vector pIRES

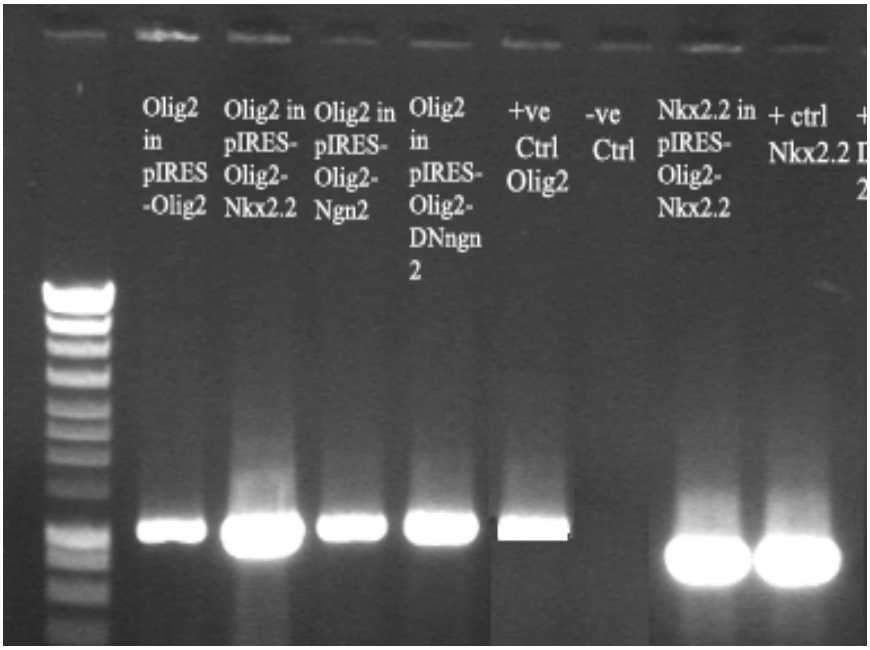


Fig 2:
PCR detection of over expression of cDNA Amplicons at MCS A or MCS B in various plamid contructs tested in mouse ES cells

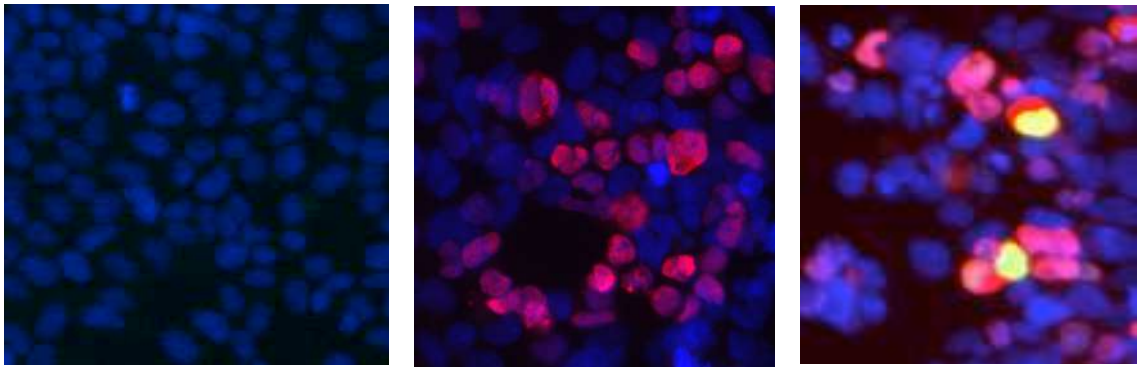


Fig3:Proof of Expression of plasmid based Olig2 and Nkx2.2 in tranfected HEK (Human Embryonic kidney cells) assessed by immuno-histochemistry.A-Control pIRES plasmid transfected cells-no expression of either Olig2 or Nkx2.2. B-Olig2 cDNA containing pIRES plasmid transfected cells-Olig2 expression.C-Olig2-Nkx2.2 double cDNA containing pIRES plasmid transfected cells- express both Olig2 & Nkx2.2

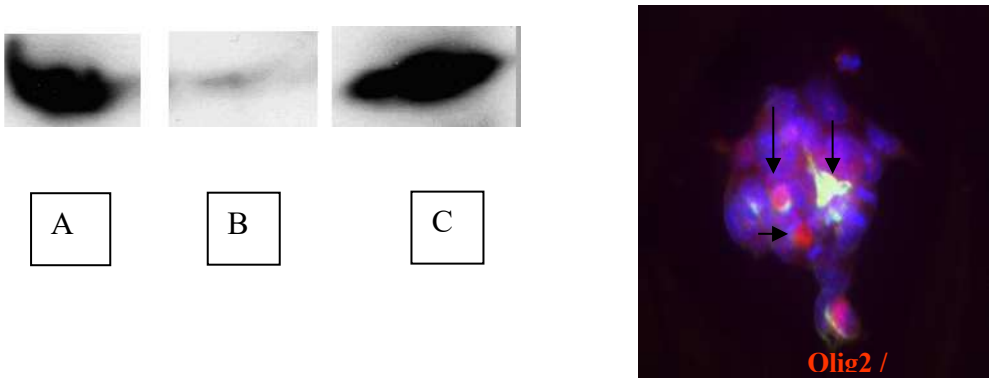


Fig 4: Immunoprecipitation using protein lysates from mouse embryonic Stem cells transfected with Olig2 and rat Nkx2.2 in pIRES-Olig2-Nkx2.2 transfected cell collections:
A- western blot confirmation of olig2 expression of 32 kDa protein band
B- control for non-specific binding: beads only
C- antigen pull-down from lysates with anti-rat Nkx2.2 and recognition of complex with anti-mouse-olig2 during co-immunoprecipitation
D-Immunohistochemical analysis of pIRES-Olig-2-Nkx2.2 transfected mouse ES cells seen to co-express Olig-2 and Nkx2.2 at 48 hours

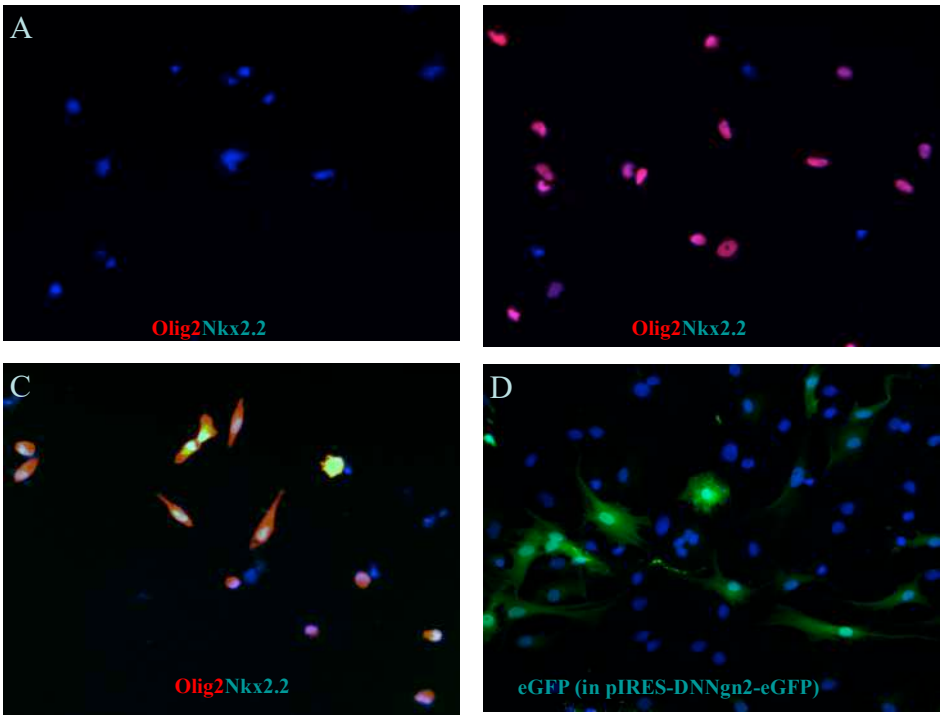


Fig 5: Expression of Olig2, Olig2 with Nkx2.2, GFP (in B locus of pIRES with dominant negative Ngn2 in A locus) in human foetal neural stem cells 48 hours post transfection with pIRES constructs: A-pIRES control, B-pIRES-Olig2,C-pIRES-Olig2-Nkx2.2, D-pIRES-ΔNgn-GFP

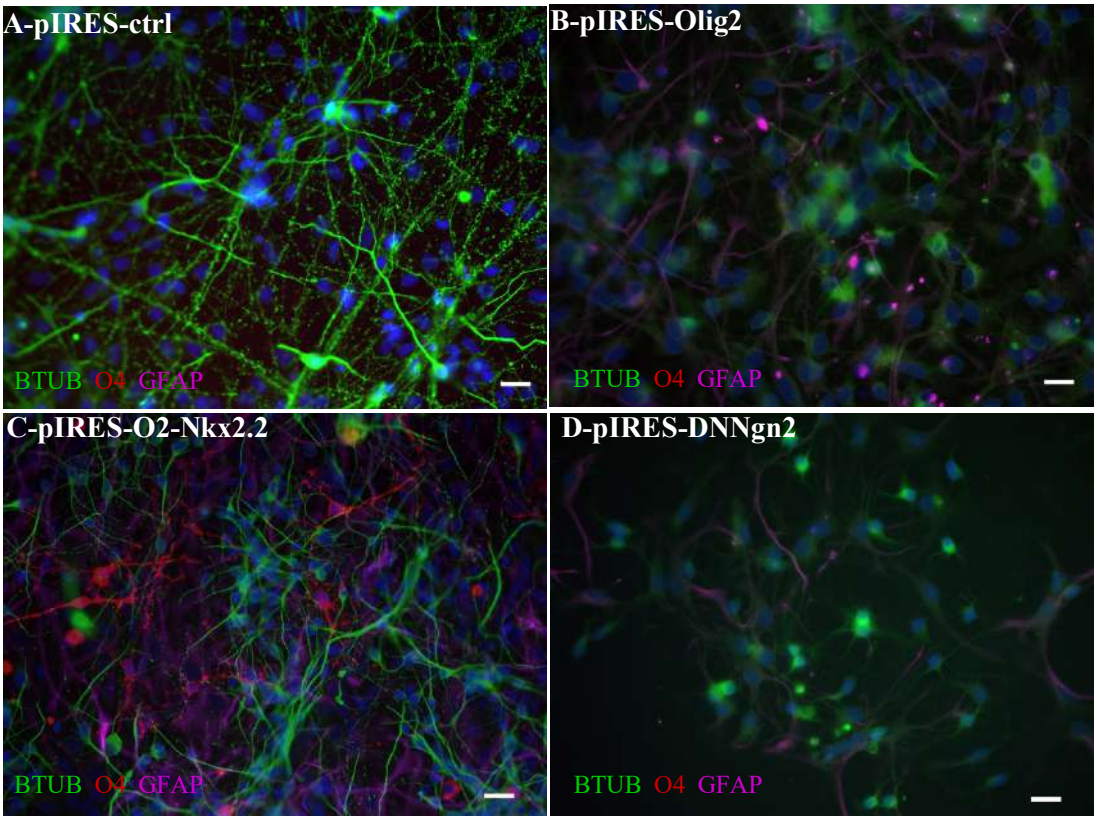


Fig 6: mES derived NPCs terminally differentiated and subjected to immuno-cytochemical analysis for lineage markers: GFAP (astroglial) ß-III-Tubulin (neural) lineages

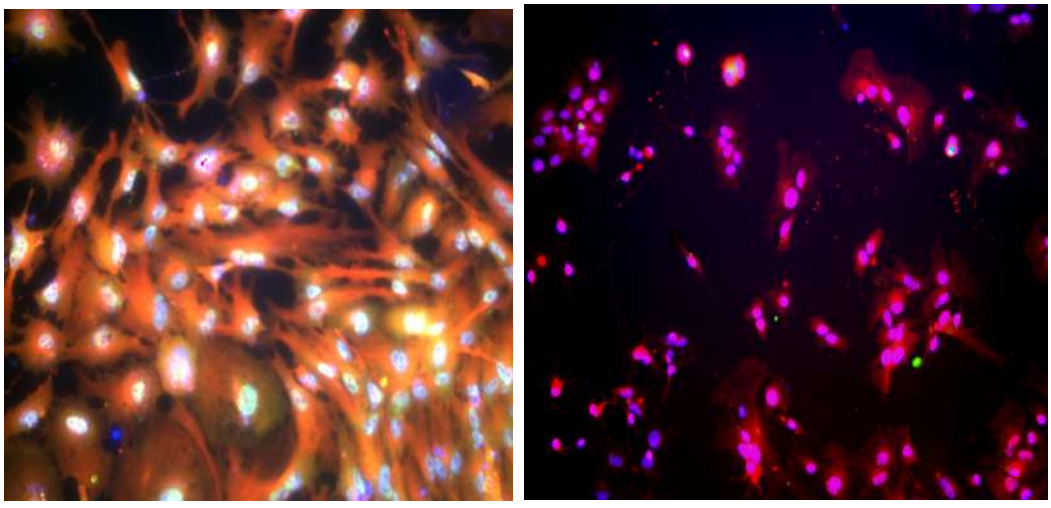


Figure 7: mES derived NPCs terminally differentiated and subjected to immuno-cytochemical analysis - additional immunocytochemical markers present in GFAP positive cells

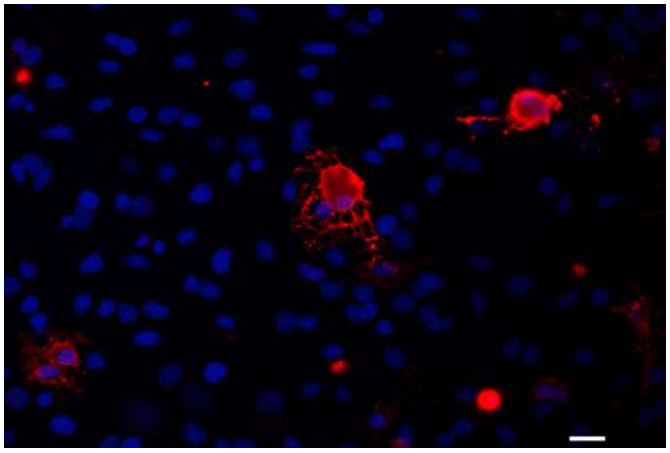
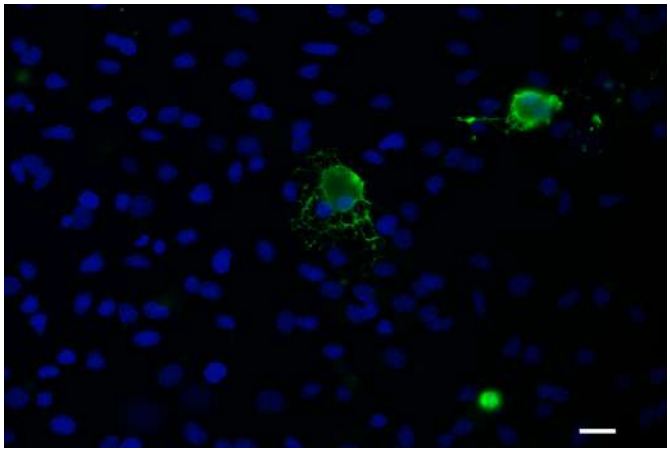


Fig 8: terminal differentiation of mouse ES cells, transfected with pIRES-Olig 2 and Nkx 2.2 constructs



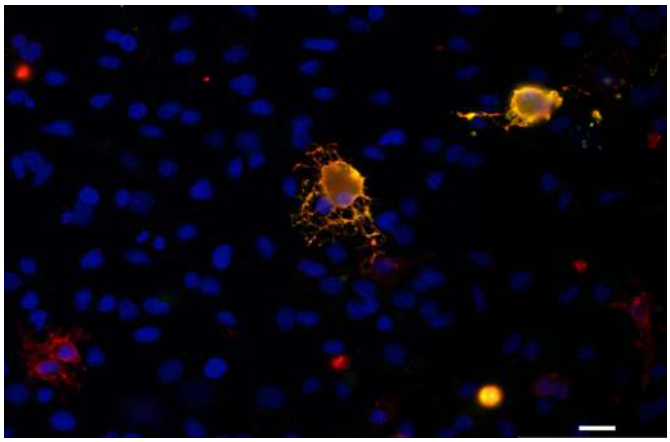
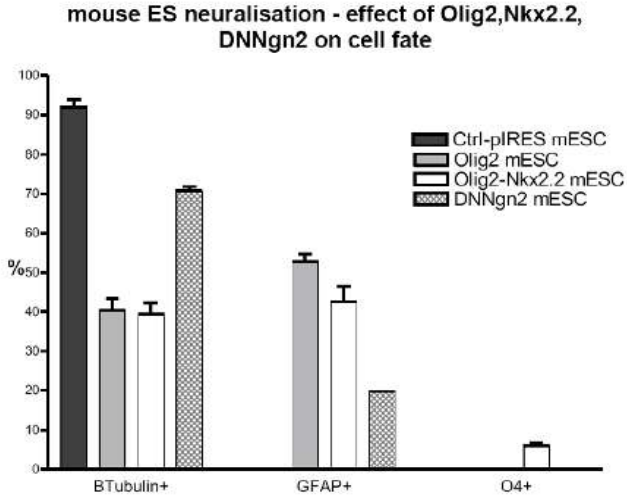


Fig 9: Demonstration of the presence of oligodendroglial markers O4 and GalC amongst terminally differentiated progeny 10 days after plate down.

Note the presence of some O4 +ve cells with GalC (larger, more arborised cells) or without GalC (smaller cells) expression.

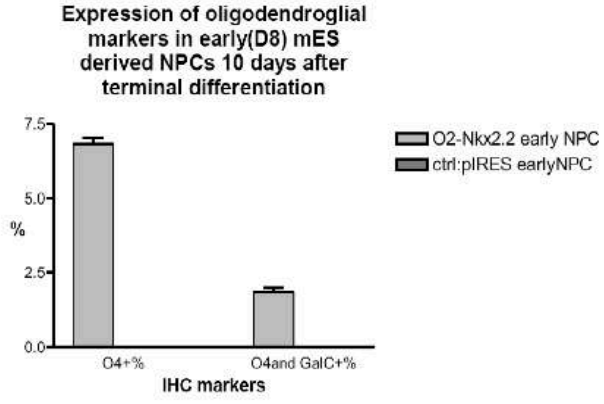
(Scale bars 25 um)



X Labels	Ctrl-pIRES mESC			Olig2 mESC			Olig2-Nkx2.2 mESC			DNNgn2 mESC		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
BTubulin+	93.92	93.67	87.50	41.44	44.94	35.05	42.51	42.22	33.61	71.29	68.85	72.03
GFAP+	0.00	0.00	0.00	50.98	51.00	56.21	45.17	47.48	36.17	19.01	19.98	19.60
O4+	0.00	0.00	0.00	0.00	0.00	0.00	6.17	7.03	5.05	0.00	0.00	0.00

Quantification of terminally differentiated mES derived from day 8 NPC, 10 days after plate down. Data for the final triplicates of experiments with fully dissociated precursors shown in table.

Fig: 10- progeny emerging from differentiating mES neural precursors by Immuno-cytochemistry



IHC markers	O2-Nkx2.2 early NPC			ctrl:pIRES earlyNPC		
	Y1	Y2	Y3	Y1	Y2	Y3
O4+%	7.2100	6.4300	6.8200	0.0000	0.0000	0.0000
O4and GalC+%	1.6100	1.8400	2.1400	0.0000	0.0000	0.0000

Quantification of O4 alone or O4 and GalC positive cells amongst the terminally differentiated NPC (day8) derived from pIRES-O2-Nkx2.2 mES cells 10 days after plate down.
(Data for triplicates of experiments is shown in table.)

Fig: 11: Oligodendroglial markers expressed by differentiating mES neural precursosr transfected with constructs

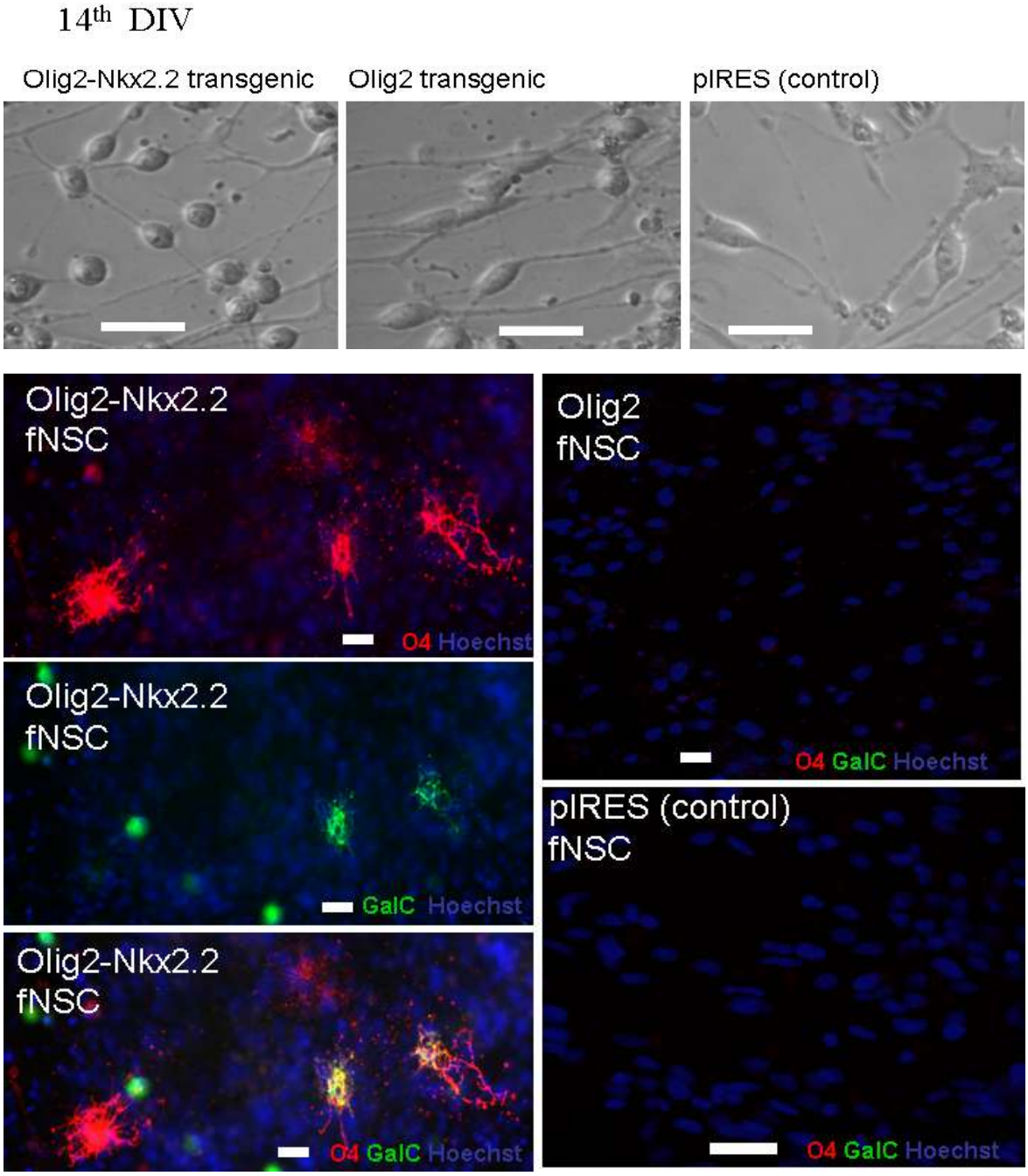


Fig: Appearance foetal NPC, after 14 days in vitro differentiation:
A-Live images.
B-Expression of oligodendroglial markers O4 and GalC, only in coverslips with the Olig2-Nkx2.2 transfected cells.

Fig: 12 : Terminal differentiation of human neural precursors transfected with transcription factor expressing plasmid constructs

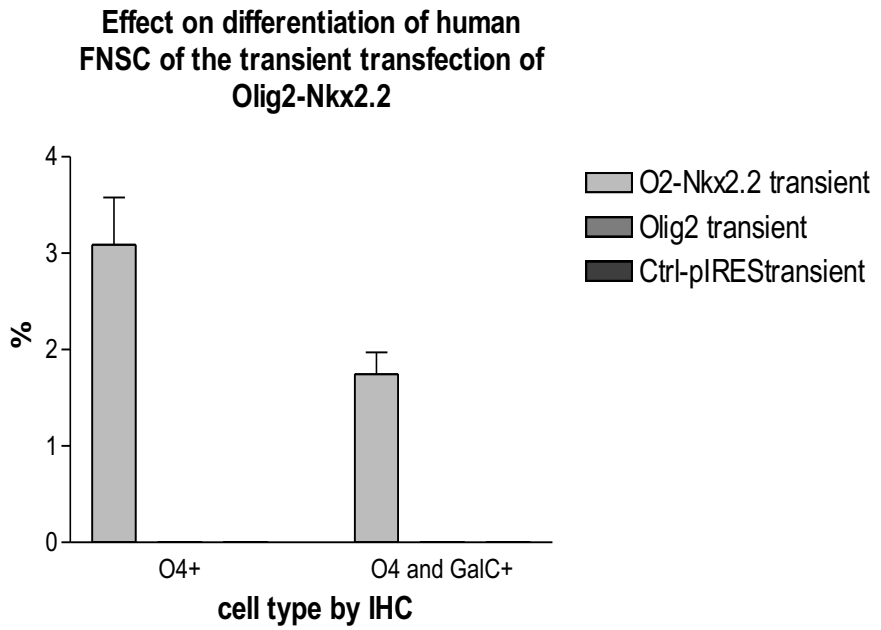


Fig 13: Immuno-cytochemical analysis of terminal cell fate and specification of human oligodendrocytes in-vitro from human foetal neural stem cells transfected with Olig-2 & Nkx2.2 over-expression.

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