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Aging, Bone Marrow and Next-Generation Sequencing (NGS): Recent Advances and Future Perspectives

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Introduction

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Review

Aging, Bone Marrow and Next-Generation Sequencing (NGS): Recent Advances and Future Perspectives

Payal Ganguly [1,†], Bradley Toghill [2] and Shelly Pathak [2,*,†]

1. Outline the challenges of the bone marrow with advancing age, and how this impacts human health

2. What we know about aging in the BM and the need for using NGS

3. NGS as a tool and how it can play a crucial role in comprehending these alterations

4. Potential, future perspectives and Challenges

Followed by a Q&A session after the presentation is completed

The aging population

In 2050, over 2 billion individuals > 65

Aging is a complex process that often results in reduced mobility, increased vulnerability to disease, poorer QOL

Aging has been identified as a state of 'chronic, low-grade, sterile inflammation' with poorly understood mechanisms

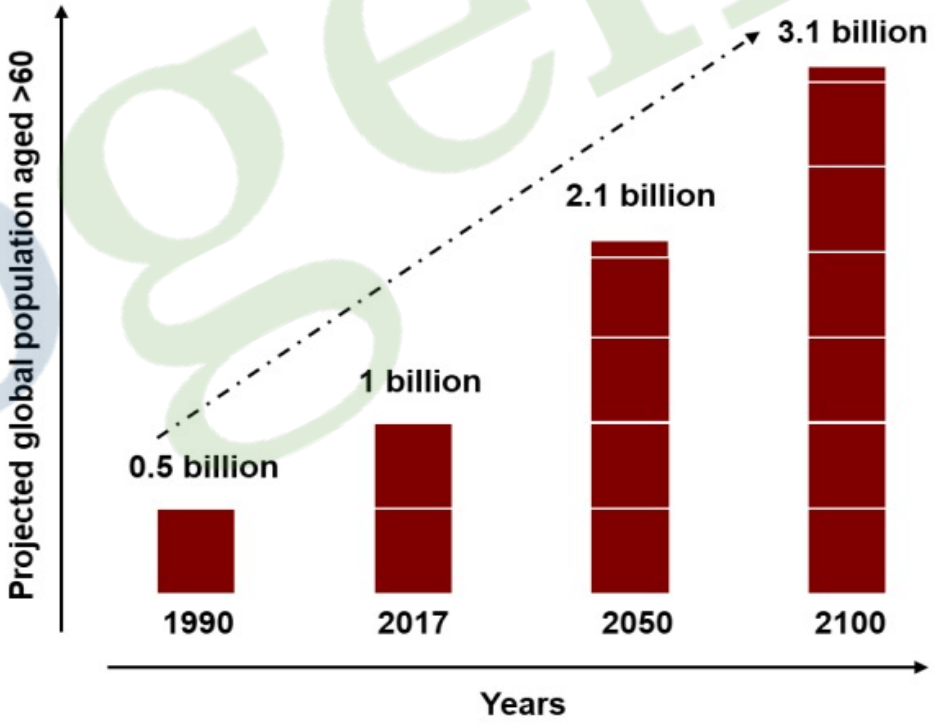


Figure 1: There is an increase in the number of people aged 60 over the next few decades until 2100. This population is estimated to reach over 3.1 billion people by 2100. Figure adapted from UN study, 2017

Aging and age-related diseases (ARDs)

This state is known as 'inflammaging' and has been associated with multiple ARDs that contribute to morbidity and mortality in the elderly

The lack of ability to fight off infections, cognitive impairment, frailty and immunosenescence are some of the most debilitating impacts of advancing age

Aging and age-related diseases (ARDs)

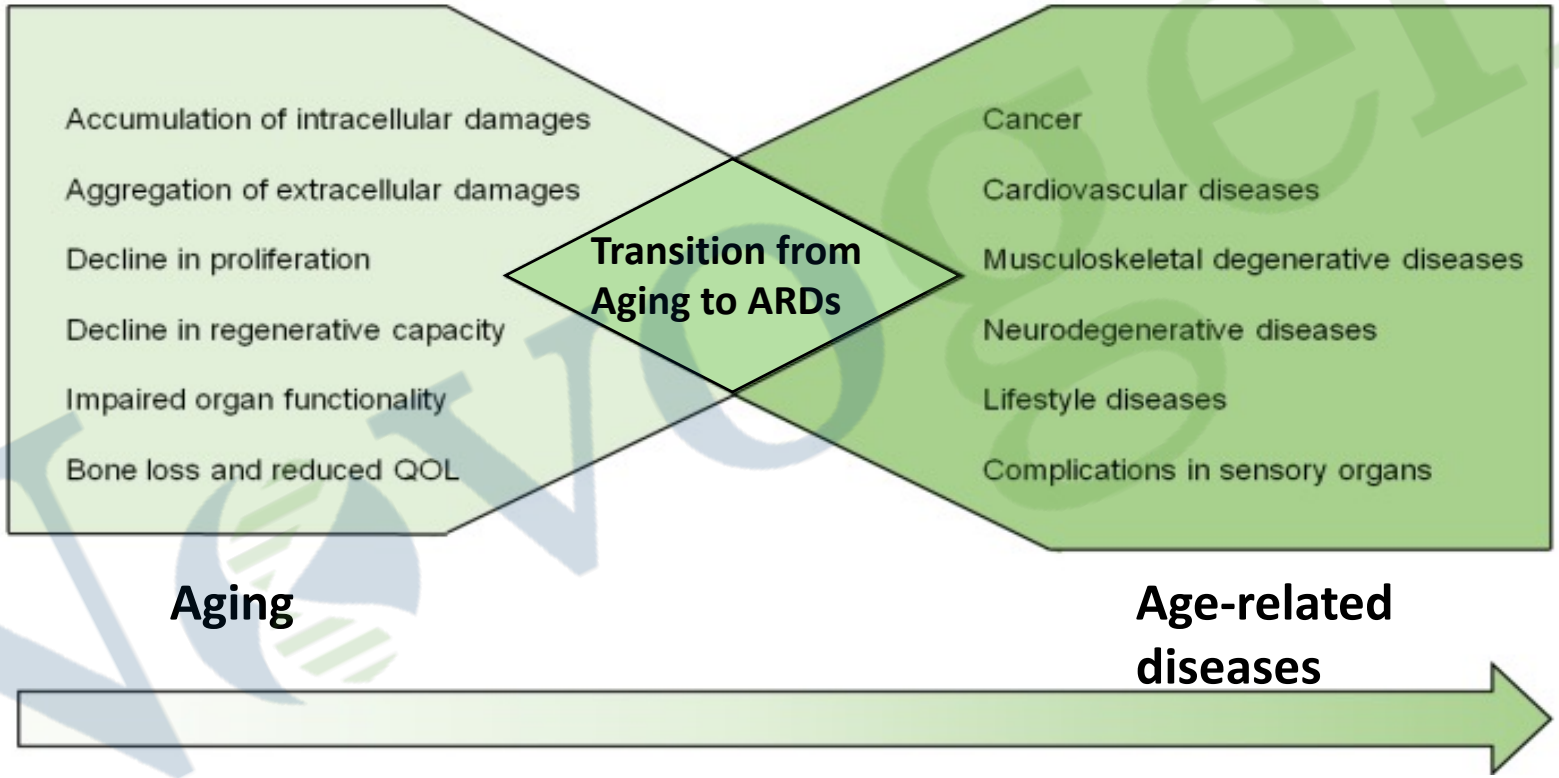


Figure 2: The link between aging and age related diseases.

The Hallmarks of Aging

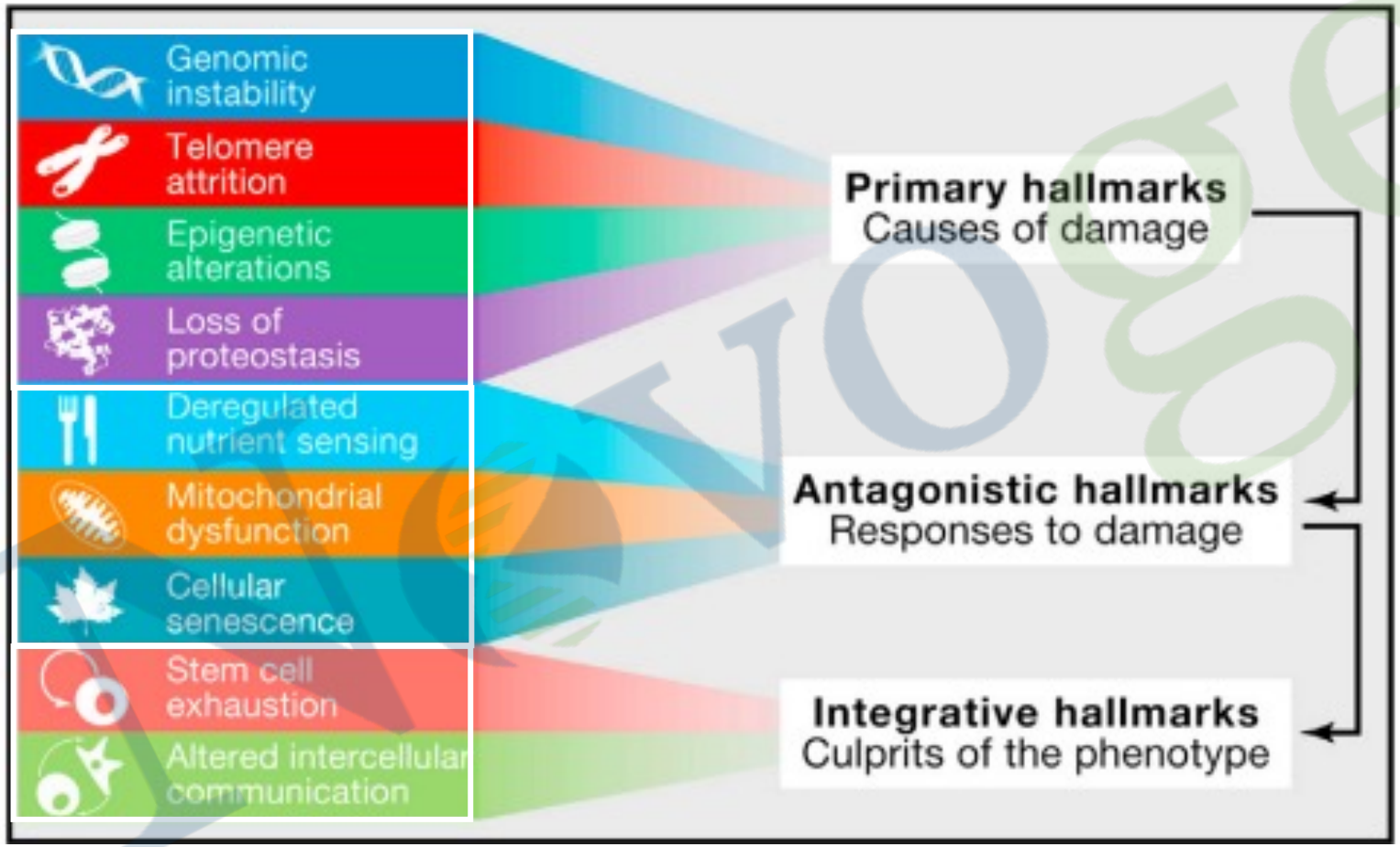
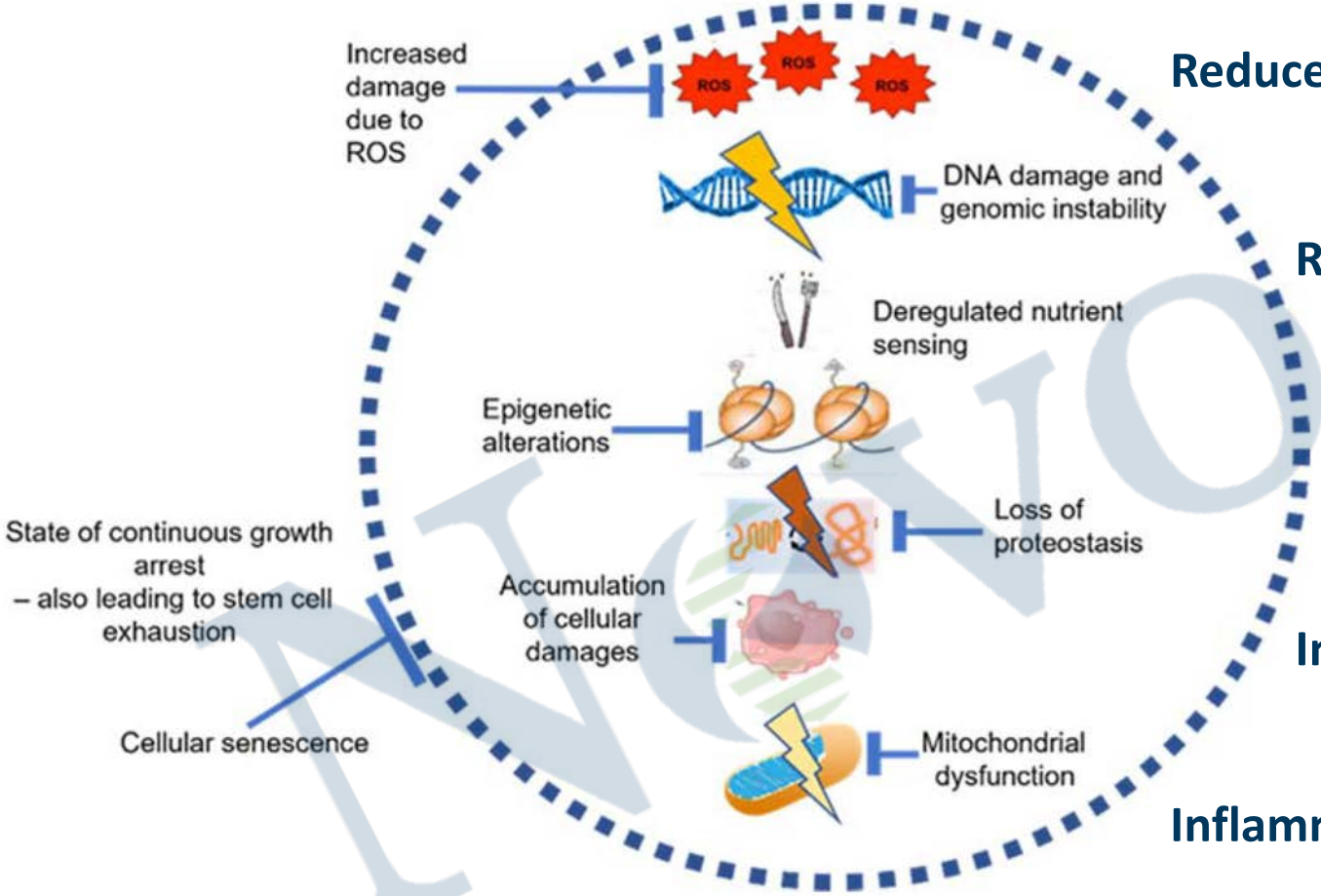


Figure 3: The scheme enumerates the nine hallmarks described by Lopez-Otin et al, 2013

The Hallmarks of aging and the bone marrow (BM)



Reduced mobility

Rigid and painful joints

Alterations in the musculoskeletal systems

Increased vulnerability to bone/joint disorders

Inflammation

Figure 4. Underlying causes of aging inside the BM, recreated and adapted using information from Lopez Otin et al. and González-Gualda et al.

The BM progenitors and cells

BM – dynamic organ with intricate 3D structure housing several cell types, growth factors (GFs), cytokines and other soluble factors

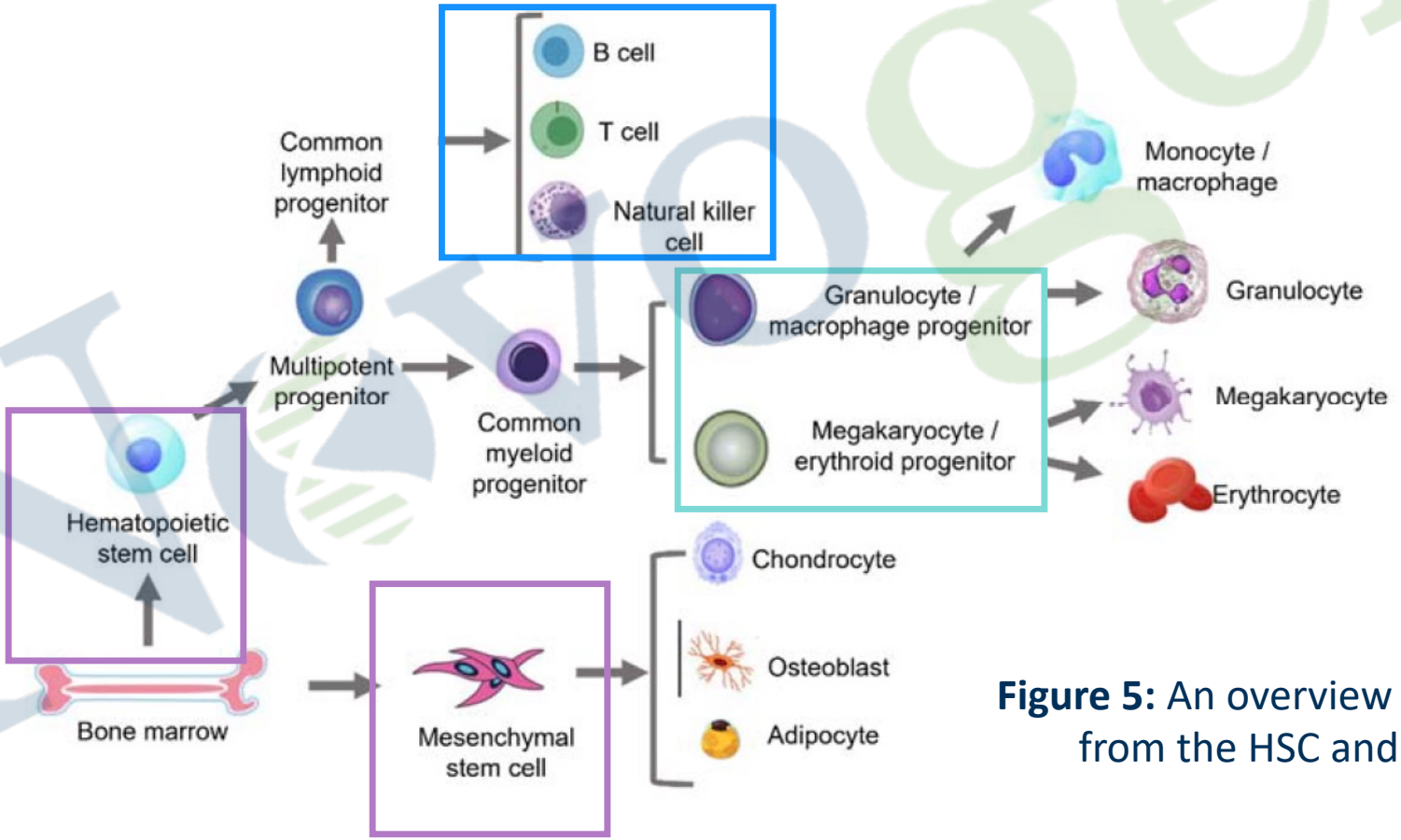


Figure 5: An overview of the lineages arising from the HSC and MSC populations

Aging in the bone marrow and need for NGS

1. **Alterations in the cellularity**

2. **Changes in proliferative capacities**

3. **Reduced functionalities and differentiation abilities**

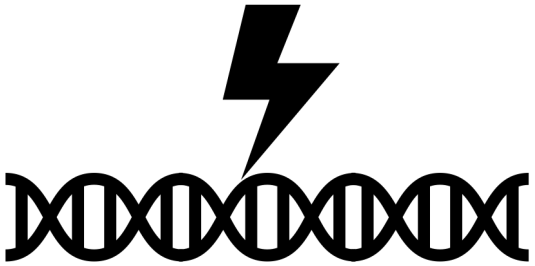
4. **Loss of bone-fat balance**

5. **Myeloid skewing**

6. **Impaired regeneration**

- Differentiation
- Proliferation
- Gene expression
- ROS
- Telomere length
- Senescence assays

DNA damage



Next generation sequencing (NGS)

What is **NGS**?

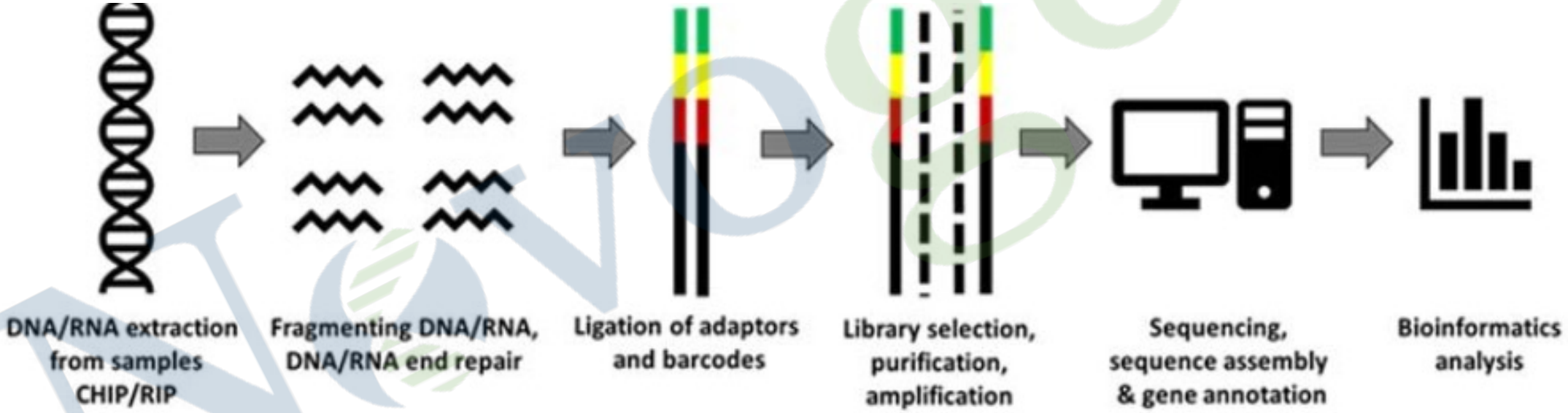


Figure 6 . Basic next-generation sequencing (NGS) workflow, figure adapted from Petric *et al*, 2015

NGS and Its Applications in Aging Studies

- NGS has been used to look at HSCs in the BM
- One of the hallmarks of aging is the accumulation of DNA damage
- Mutated stem cells within the BM, can lead to an increased risk of haematological malignancy development
- For example, each decade, an average of 1.3 ± 0.2 somatic mutations are developed per HSC, and age-related haematopoietic clones in individuals over the age of 55 is a relatively common finding
- These mutations may not have any functional affects on the body

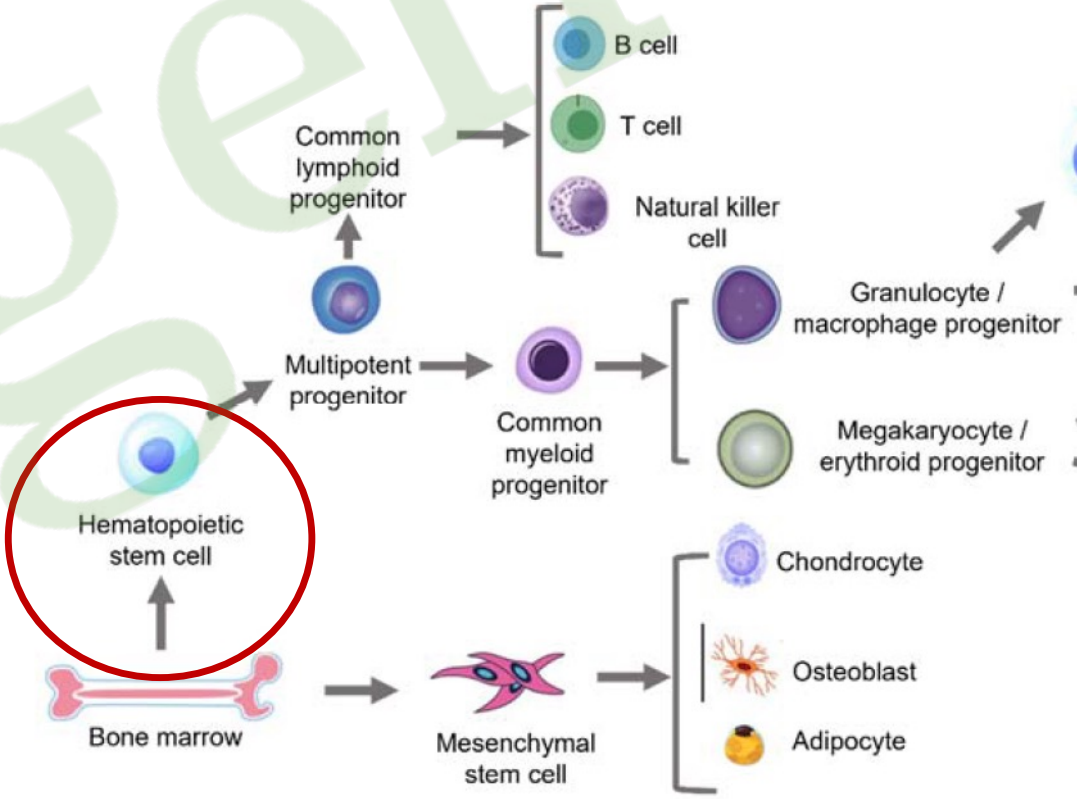


Figure 7: An overview of the lineages arising from the HSC and MSC populations

NGS and Its Applications in Aging Studies

- Some mutations make cells independent of specific external growth factors, or develop resistance to these, causing uncontrolled expansion

- In order to understand this properly, NGS has allowed scientists to probe the genome, exome and transcriptomic profiles of cells residing within the BM in an attempt to understand the underlying molecular mechanisms.

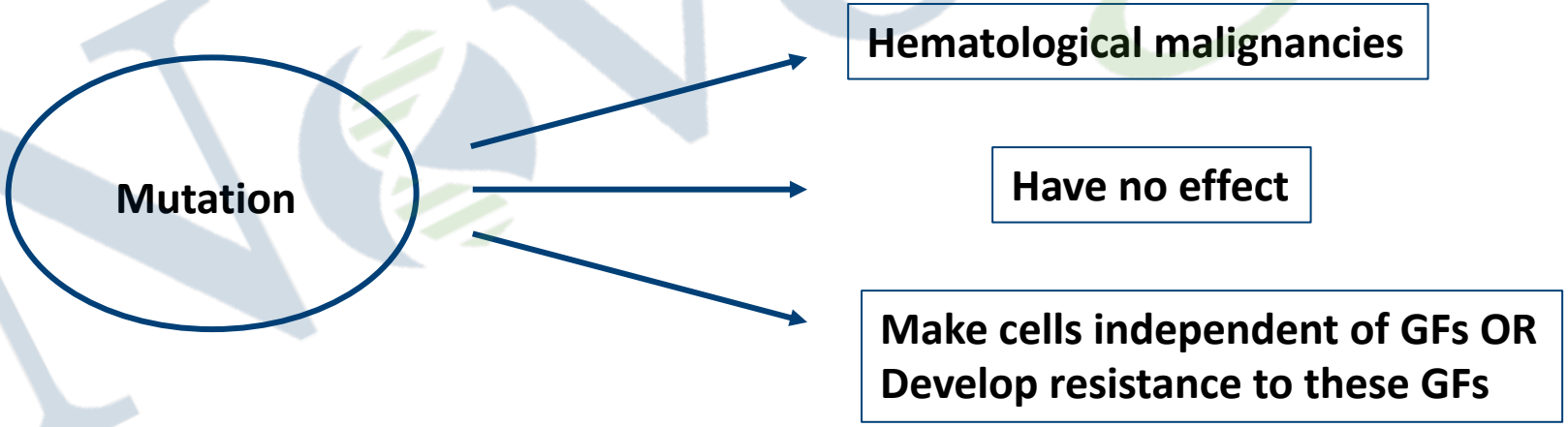


Figure 8: The impact of mutations in HSCs

Targeted Sequencing Approaches

- Targeted sequencing focuses on specific areas of the genome
- Main methods of targeted NGS include hybridisation capture and amplicon sequencing

Feature	Hybridisation capture	Amplicon sequencing
Starting amount	>400ng	200ng
Sensitivity	1%	5%
Cost per sample	Various	Generally cheaper

Table 1: Features of TRS

Targeted Sequencing Approaches

Example study of Targeted Sequencing (Pathak et al, 2019):

Exploratory Study of MYD88 L265P, Rare NLRP3 Variants, and Clonal Hematopoiesis Prevalence in Patients With Schnitzler Syndrome

HSC populations → Examined → 1 SchS patient demonstrated non-sense mutation in *STAG2* with a low variant allele fraction

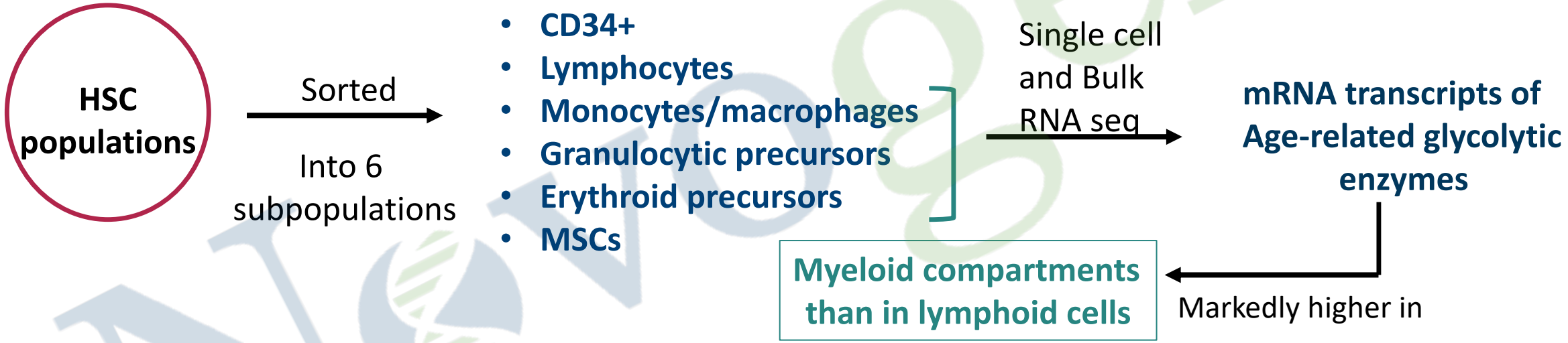
Panel of 28 genes Associated with aging including development of clonal haematopoiesis and myelodysplastic syndrome

Conclusion of the study: Clonal haematopoiesis was not associated with Schnitzler Syndrome (SchS) patients—therefore ruling out mutations associated with aging in this bone marrow disorder

ScRNA and Bulk RNA-Seq

Examination of isolated cellular populations through looking at its transcriptome

Example 1 (Hennrich et al, 2018):



This sequencing was conducted using the Switch Mechanism at the 5' end of the RNA templates (SMART-seq); a single-cell protocol developed to enable a complete genome coverage, thereby permitting the detection of alternative transcript isoforms and SNPs

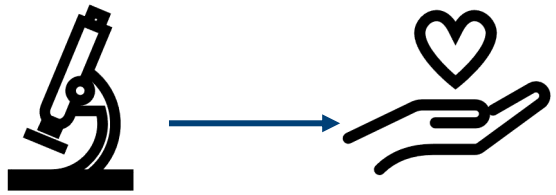
Conclusion of the study: Aging triggers changes within the BM niche, specifically reducing the pathway functions involved in human haematopoietic stem and progenitor cell homing

The Future of NGS in Aging Studies—Potential & Perspectives

1. Use of NGS for BM aging in progenitor and immune cells from human donors of wide age range (18–90)

2. Comparative investigations of transcriptome reads between healthy old and donors with ARDS → narrow down on genetic factors present in the healthy elderly but potentially exacerbated in donors with ARDs

3. Approaching the ever-dynamic BM and its aging cells will help us understand the differences in the genome with healthy aging AND help with the early diagnosis of various ARDs that are commonly observed in the BM



4. These investigations could aid in slowing the progression of debilitating diseases like cancer and enhance the QOL of patients through more personalised therapies

Early diagnosis

Enhanced QOL, Personalised medicine

The Future of NGS in Aging Studies—Challenges

Major challenges

1. Technical challenges

- **Poor cellularity**
- **Low quality DNA/RNA**
- **Variation of tissue sources**

E.g. Several cell populations (B and T cells) decline with advancing age→ extracting sufficient quantities of genetic material from samples of older donors will pose challenges during the library preparation for sequencing

2. Analytical challenges

Vast amount of data generation

- One alignment file (BAM file); accounting for 30× the coverage of the human genome would approximately generate 90–95 Gb of data
- Applying this to simply 10 samples will generate nearly 1 Tb of data

Solution: Encourage the sharing /deposition of such NGS data in public domains. The National Center for Biotechnology Information (NCBI) has an ever-growing bank of such data

Conclusions

1. Aging and ARDs reduce QOL of the elderly and present socio-economic challenges, globally
→ Aging research needs to move at a faster pace than our aging population

2. The last couple of decades have increased our understanding of age-related changes within the BM. However, novel techniques providing data at the genetic level are essential for discovering pathways towards the aging BM

3. Focussing on gene panels aimed at the proliferation, differentiation, migration and homing of these cells with advancing age will also add to our knowledgebase of aging BM

4. NGS provides us with large amount of data at the genetic level for the identification of new pathways which is ideal for evaluating age-related changes in the BM

5. This will help us underpin the pathways involved in the transition from healthy aging to ARDs→ Allowing for applications in early diagnosis and enhanced care and QOL of the elderly

Thank You

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