Supplemental Information

Clonal hematopoiesis: a manifestation of somatic DNA damage

Age-related clonal hematopoiesis is a prevalent, pre-cancerous state that results from the expansion of hematopoietic stem cell clones that acquire any one of a number of "driver" gene somatic mutations. The expanding clones increasingly give rise to progeny leukocytes that harbor the mutation, and this can alter their phenotypes and contribute to disease. Clone sizes typically range from a small fraction of less than 0.5%, and can only be detected by deep error-corrected sequencing [121], to a condition where the repertoire of an individual's blood cells can be almost entirely derived from two related HSPC clones in a super-centenarian [122]. This condition is age-associated and prevalent in the elderly. Depending on the methodology employed, estimates for clonal hematopoiesis range from 10% of those older than 70 years of age [123] to more than 50% for subjects older than 85 years [124]. Numerous recent studies have associated clonal hematopoiesis with mortality due in large part to increased cardiovascular disease [125]. Molecular mechanisms by which these somatic mutations alter the immune system and contribute to age-associated disease have been defined [126-130]. These studies show that clonal hematopoiesis leads to the overactivation of inflammatory responses in a gene-specific manner and contributes to "inflammaging" [131]. While it is clear that different mutant driver genes confer distinct phenotypes to their progeny leukocytes within the clone, experimental studies have also identified common features between these different forms of clonal hematopoiesis. Most notably, overactivation of the IL-1β and/or IL-6 signaling pathway is observed among different driver gene-mediated clones. At the level of the hematopoietic stem

cell, it is believed that these somatic mutations confer enhanced responses to inflammation and protection from inflammation-induced damage [132]. Because these same mutations lead to systemic elevations IL-1β and IL-6 levels, a positive feedback loop is created that leads to a selective advantage for mutant cell expansion in the bone marrow niche. These experimental findings relating clonal hematopoiesis to excessive inflammation via IL-1β and/or IL-6 have been validated in clinical analyses utilizing the TOPMed, UK Biobank [133], and CANTOS cohorts [134].

The age-dependent appearance of mutant clones is not limited to the hematopoietic system. Studies using deep DNA sequencing have discovered that the phenomenon of accumulation of stem cells with driver mutations and expansion of clones occurs in multiple tissues, particularly those with high rates of cell turnover, including skin and liver [135]. This leads to increasing somatic mosaicism with aging. The consequences of this somatic mosaicism remain to be established.

Supplemental Table 1. Measures to establish somatic DNA damage and its cellular effects. Broad categories are listed in the cells. Methods are in bold. Targets characterized by each method are listed. Cells in blue indicate methods that can be readily adapted to relatively small samples of human blood.

Genotoxic stress	**Immunoblot**	**IHC/IF**	**qPCR**		
	p53/p-p53	p53	p53 targets		
	p21	p21			
	ATM/p-ATM	γH2AX			
	γH2AX	53BP1			
Cell fate	**Immunoblot**	**IHC/IF**	**qPCR**	**Stain**	**ELISA**
Apoptosis	Cleaved caspase-3	Cleaved caspase-3			
	Annexin V	Frag-EL			
		Annexin V			
		TUNEL			
Senescence	p16	p16	p16	SA-βGal	SASP
	p21		SASP		
	γH2AX				
	LaminB1				
	HMGB1				
Oxidative stress	**ELISA**	**Mass Spec**	**EPR**	**Immunoblot**	**Biochemical assay**
Lipids	4-HNE adducts	oxidized lipids			
Proteins	protein carbonyls	protein carbonyls			
		Hgb adducts			
DNA	8-oxodG	8-oxodG			
ROS			superoxide anion		DHE
			ascorbate radical		
anti-oxidants				catalase	catalase activity
				SOD1	SOD1 activity
				SOD2	SOD2 activity
					GSH/GSSG
pro-oxidants				NADPH oxidase	NADPH oxidase activity
				XO	XO activity
DNA damage	**LC-MS/MS/MS**	ELISA	Immunoblot		
	8-oxodG	8-oxodG	M_1G		
	cyclopurines				
	ethenoadducts				
	adductome				