CHARACTERIZATION OF PROGRESSION OF GENOMIC CHANGES DURING CLINICAL COURSE OF AML

EXELIXI

ALEX CHANG
MARTIN JADERSTEN
EMILIA LIM
LISA RAEBURN
RODRIGO GOYA
MARTIN KRZYWINSKI
MARIA MENDEZ-LAGO

BC Cancer Research Center
Genome Sciences Center

NATURE OF ACUTE MYELOID LEUKEMIA

role of bone marrow stem cells

insensitivity to chemotherapy

ACUTE MYELOID LEUKEMIA

bone marrow malignancy

rapid growth of abnormal white cells which accumulate and interfere with production of normal blood cells

1,400 cases per year in Canada, 5-year survival 15-70%

INEFFECTIVE CLINICAL COURSE

50% of patients relapse after chemotherapy and require bone marrow transplant

OPPORTUNITY FOR IMPROVING OUTCOMES

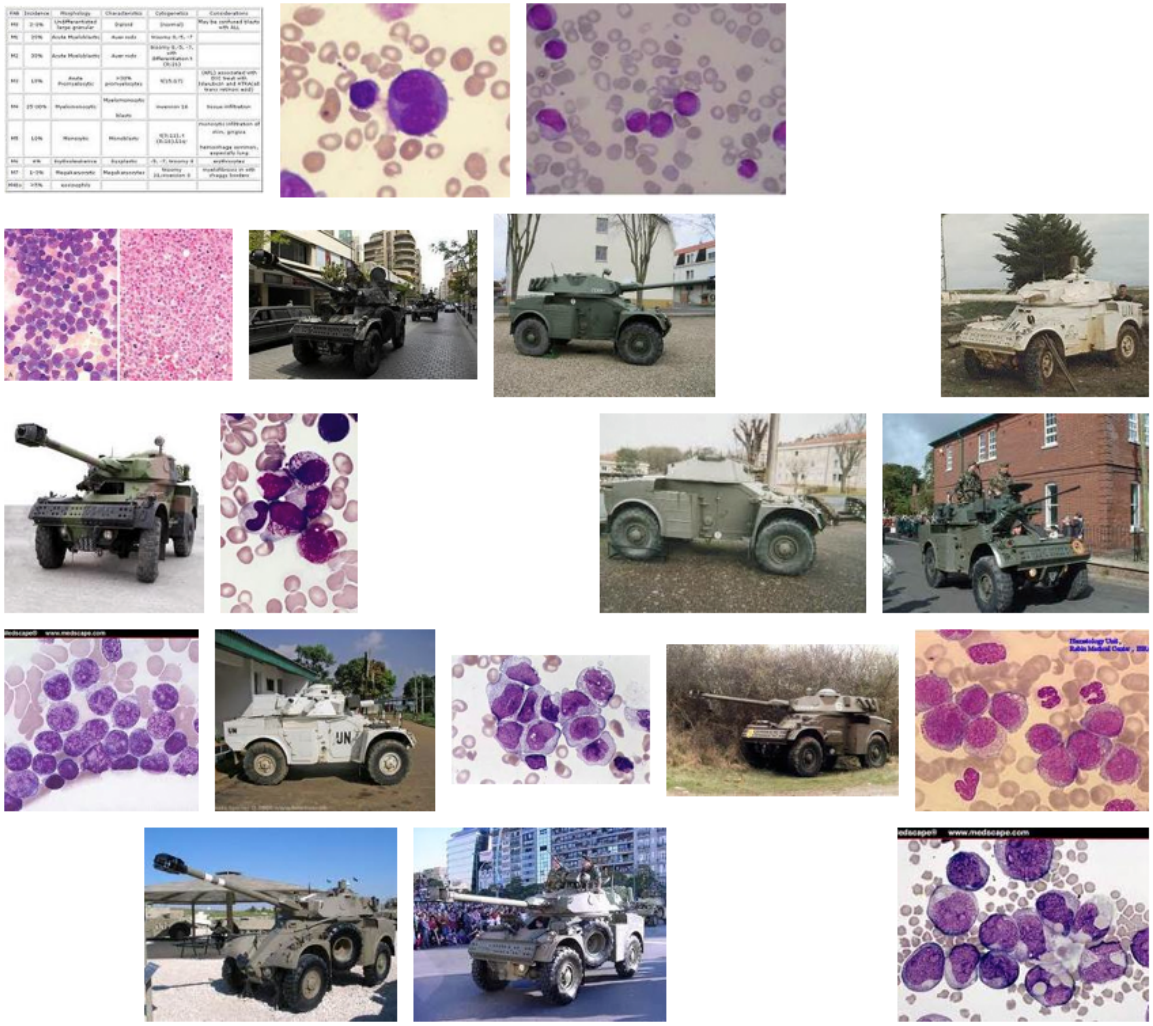
little is known about reasons for relapse in normal karyotype AML

MODEL FOR GENOME EVOLUTION AND PROGRESSION

bone marrow cells essentially eradicated during chemotherapy

population bottleneck amplifies effect of clonal expansion conferring resistance

WHAT DOES AML LOOK LIKE?



Google image searching for AML retrieves slides of cells … and light-armoured vehicles (Panhard AML-90), already known to be ineffective in battle against AML

CHEMOTHERAPY RESISTANCE – BONE MARROW STEM CELLS

bone marrow stem cells contribute to resistance to chemotherapy

quiescent

express transport proteins that expel toxins

RESISTANT SUBCLONES

populations of cells with mutations that confer resistance

resistant subclones may be present at diagnosis or created by chemotherapy

differential mutational frequency may exist in stem cell compartment

RELEVANCE

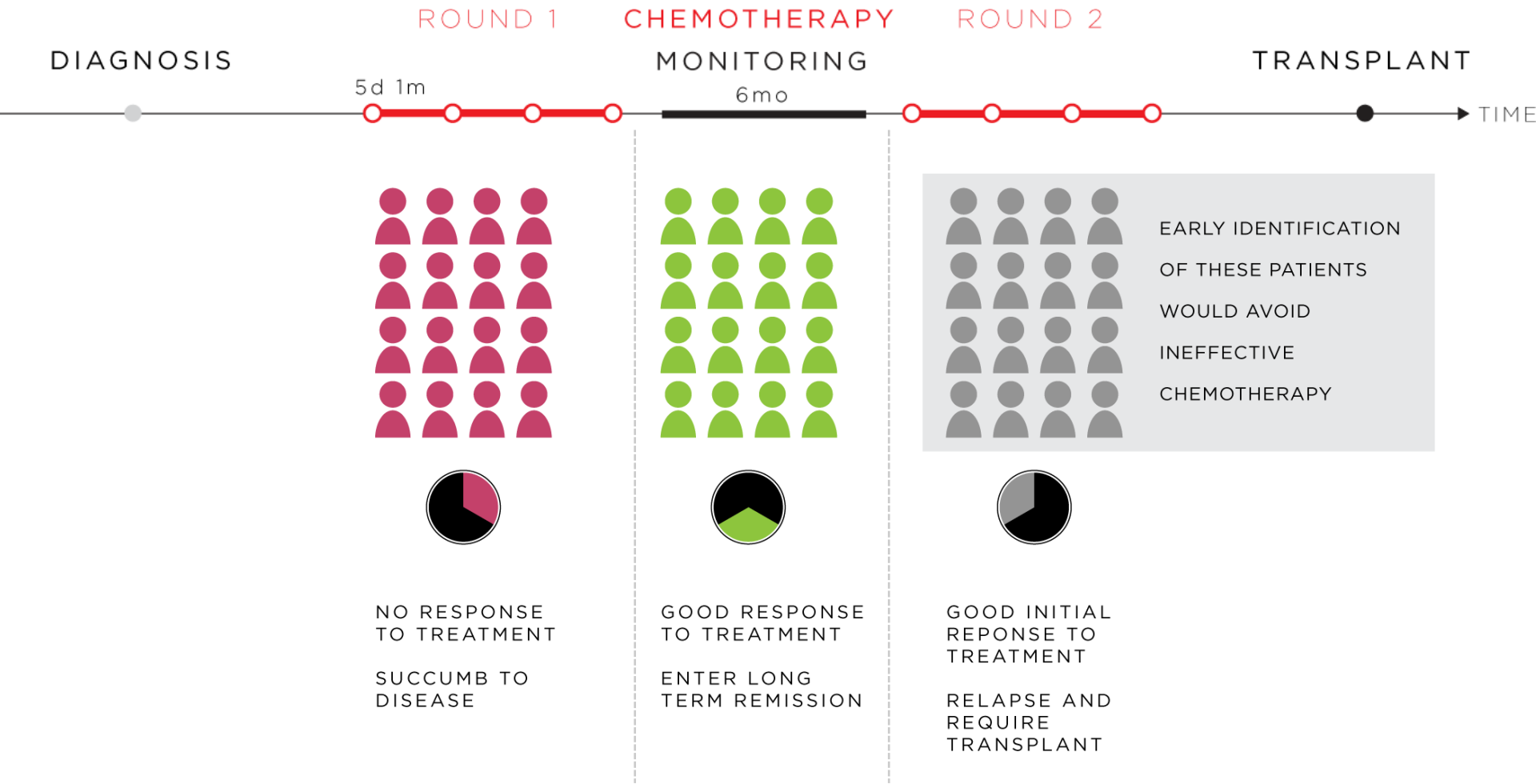
IMPROVED TREATMENT MANAGEMENT

current prognosis methods lack specificity

standard treatment protocol is inefficient for many patients

CURRENT TREATMENT METHODS

patients receive a combination of chemotherapeutics



CURRENT PROGNOSIS METHODS

AML samples characterized with Sanger-type sequencing at diagnosis

detection limited to ~20% allele frequency

insensitive to rare subclones

no progression monitoring, to identify emergent resistant subclones

OBJECTIVE

IMPROVE AML TREATMENT OUTCOME

characterize molecular evolution of disease during treatment

correlate genomic alterations with outcome

BETTER DISEASE TYPE CLASSIFIER

identify prognostic markers and therapeutic targets

SAMPLE COLLECTION AND CHARACTERIZATION

collect and separate bone marrow samples

sequence samples to identify new mutations

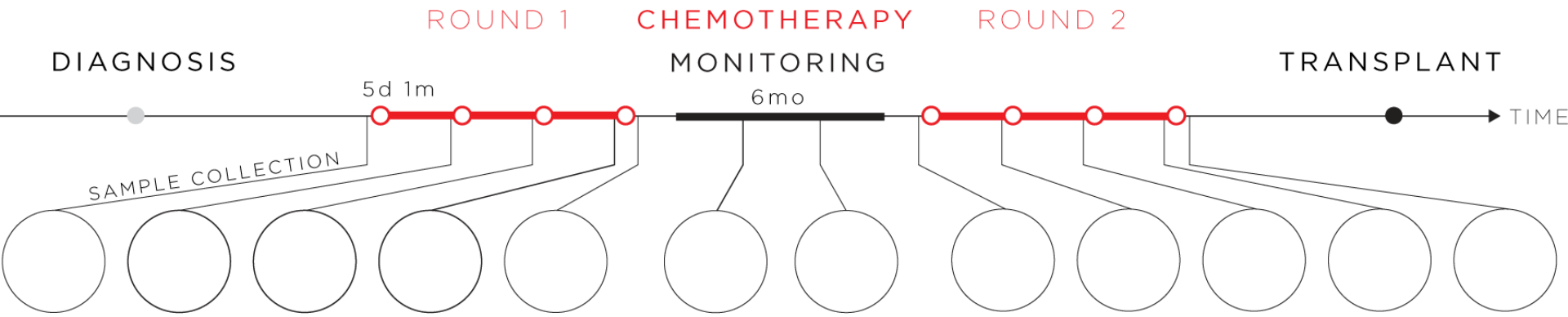
assemble genomes and transcriptomes

interrogate mutations in samples with low cell count using PCR

SAMPLE COLLECTION

follow 30 normal karyotype AML patients from time of diagnosis

collect bone marrow samples prospectively during treatment and monitoring



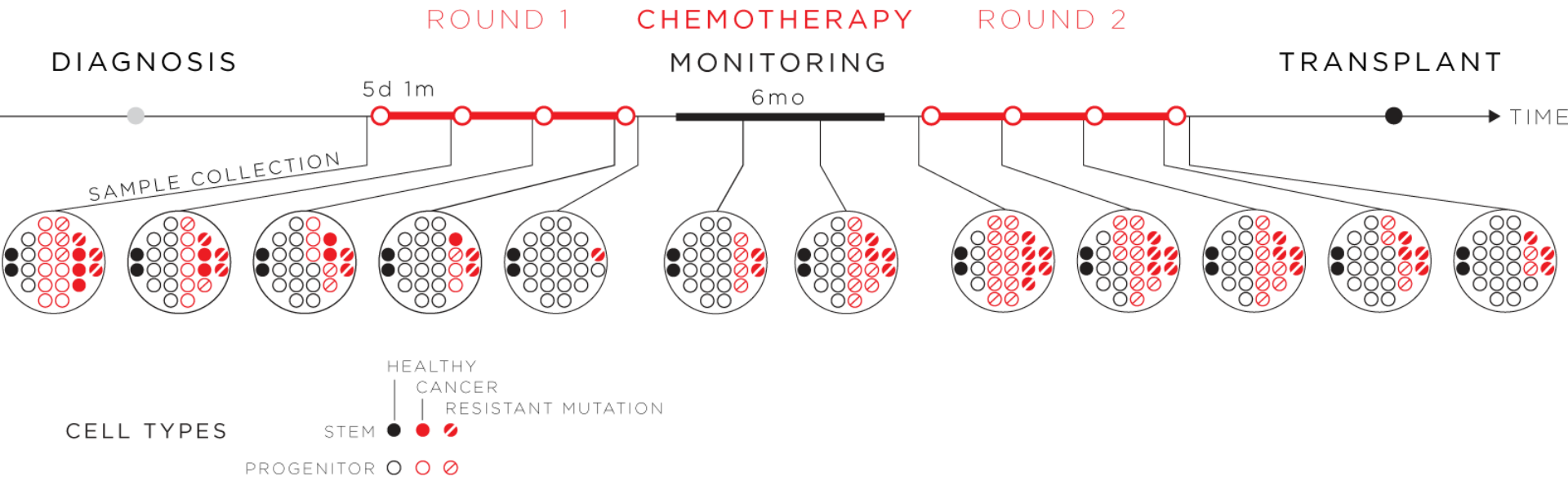
collect skin biopsy for matched normal

WHAT DO WE EXPECT FROM RELAPSE CASES?

cancer cell population drastically reduced during chemotherapy

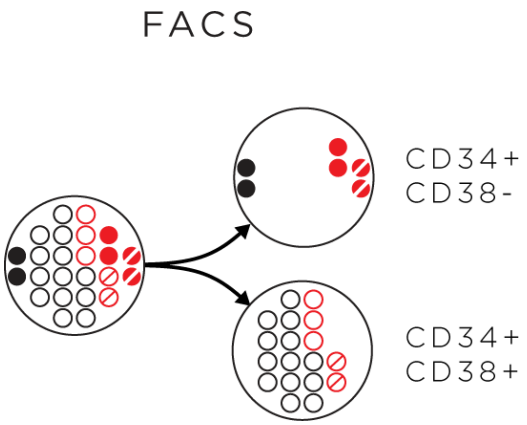
population increases during monitoring

rare resistant subclones dominate



SEPARATE CELLS AND CREATE LIBRARIES

apply flow cytometry to separate stem cells from progenitors



generate DNA and RNA libraries for WGSS and WTSS

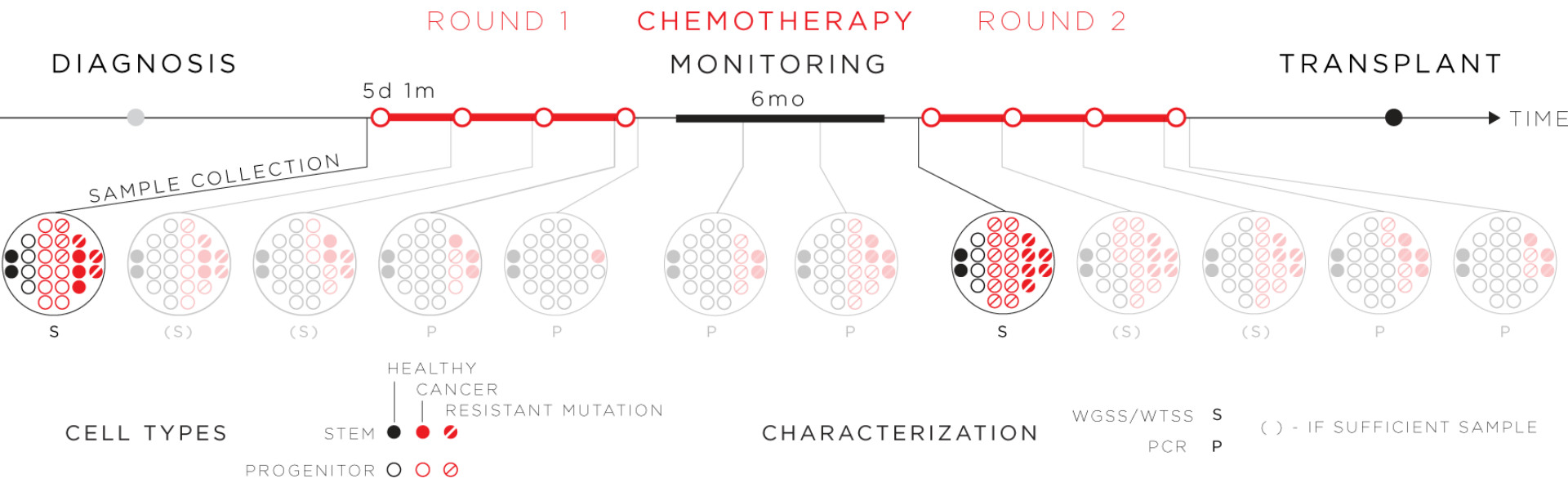
ROLE OF STEM CELL FRACTIONATE

genomic and gene expression landscape of stem cells will be different

CHARACTERIZE SAMPLES

apply HiSeq to samples with sufficient cell count

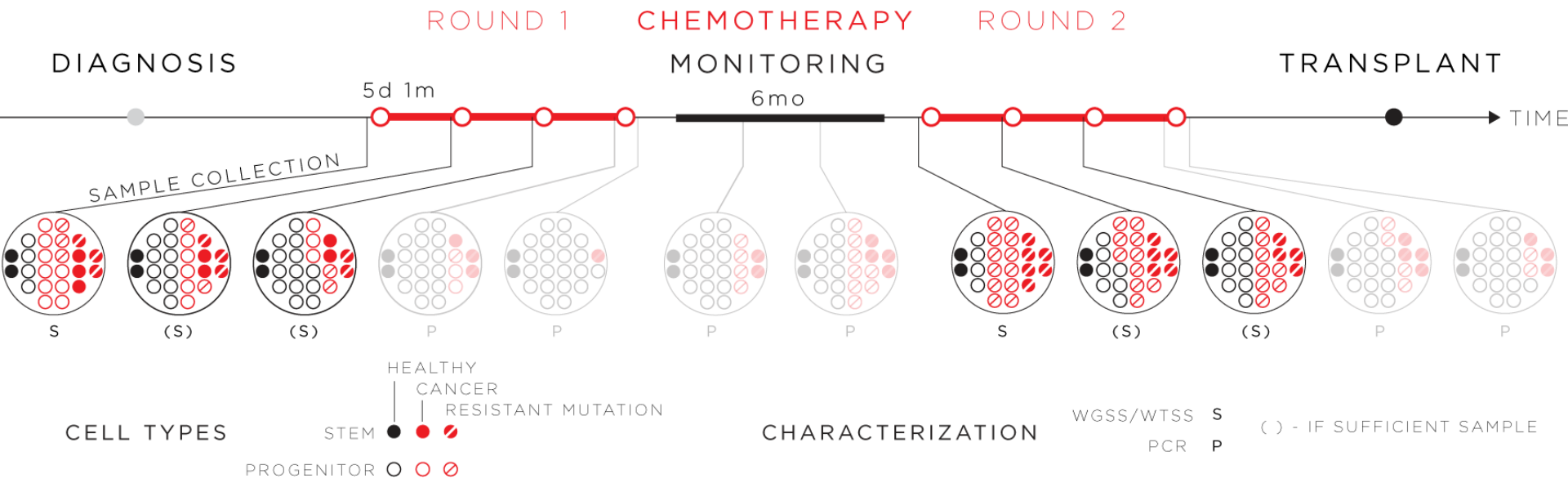
- at diagnosis
- at relapse



CHARACTERIZE SAMPLES

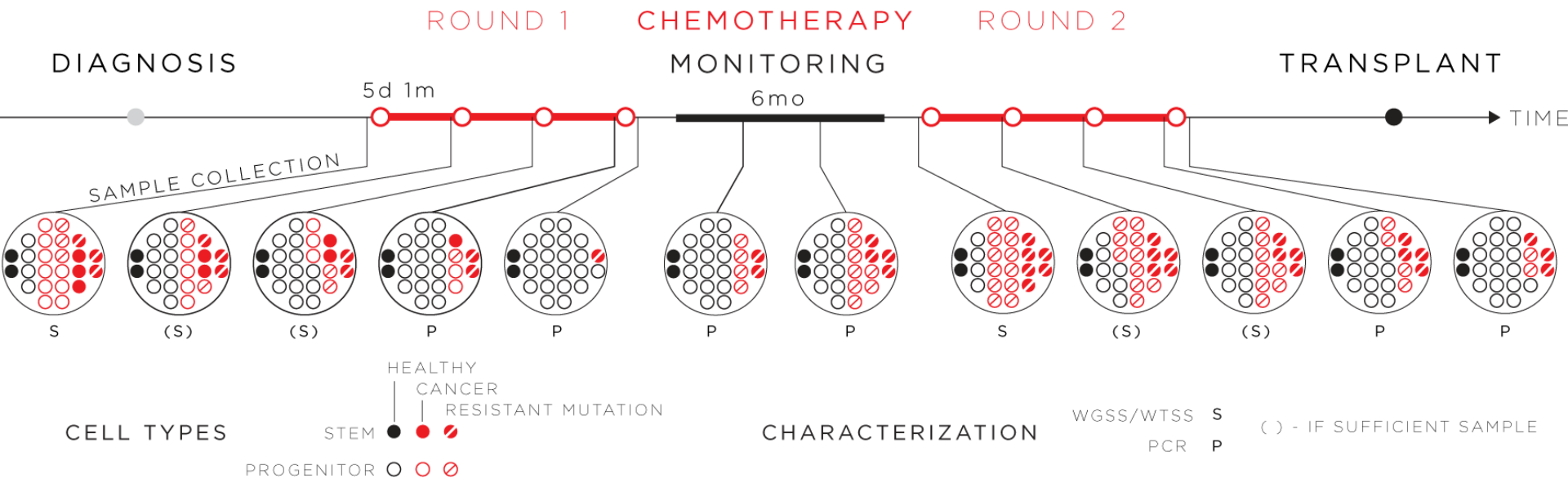
samples at 1st/2nd treatment in each round may have sufficient cell content

if so, sequence these



CHARACTERIZE SAMPLES

interrogate remaining samples with PCR



REFERENCE ALIGNMENT

align with BWA
identify SNV with samtools/SNVMix
use pair-end reads to map rearrangements
identify alternate splice sites with Tophat, HMMSplicer
perform expression and alternative expression analysis

DE NOVO ASSEMBLY

assemble genome with ABySS
assemble transcriptome with Trans-ABySS
identify fusion transcripts with deFuse

CELL COUNTS AND ALLELE FREQUENCY

LEUKEMIC STEM CELLS

expect 0.1-10 x 10^6 LSC per sample at diagnosis and relapse

this is sufficient DNA/RNA sequencing

samples from which sufficient genomic material cannot be collected will be interrogated by PCR

TARGETED DEEP SEQUENCING

during treatment, malignant cells will be regenerated by normal cells

FACS may not reliably separate leukemic and normal cells due to novel phenotypes (new markers)

to identify presence of rare alleles, we will apply deep targeted sequencing based on WGSS results

IMPACT

REDUCE THERAPY COST AND IMPROVE OUTCOMES

identification of new prognostic markers

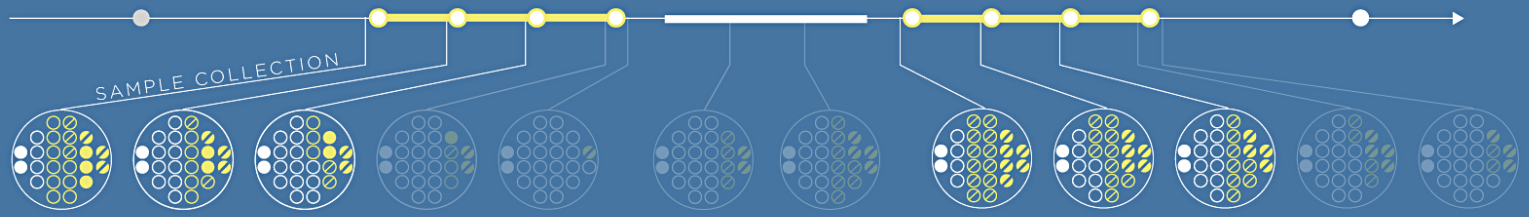
afford earlier detection of high-risk patients to fast-track transplants

discovery of drug targets informed by novel, validated and functionally relevant aberrations

extend to functional characterization and validation of AML mutations in cell lines and animal models

BUDGET

SEQUENCING AND VALIDATION SCHEDULE



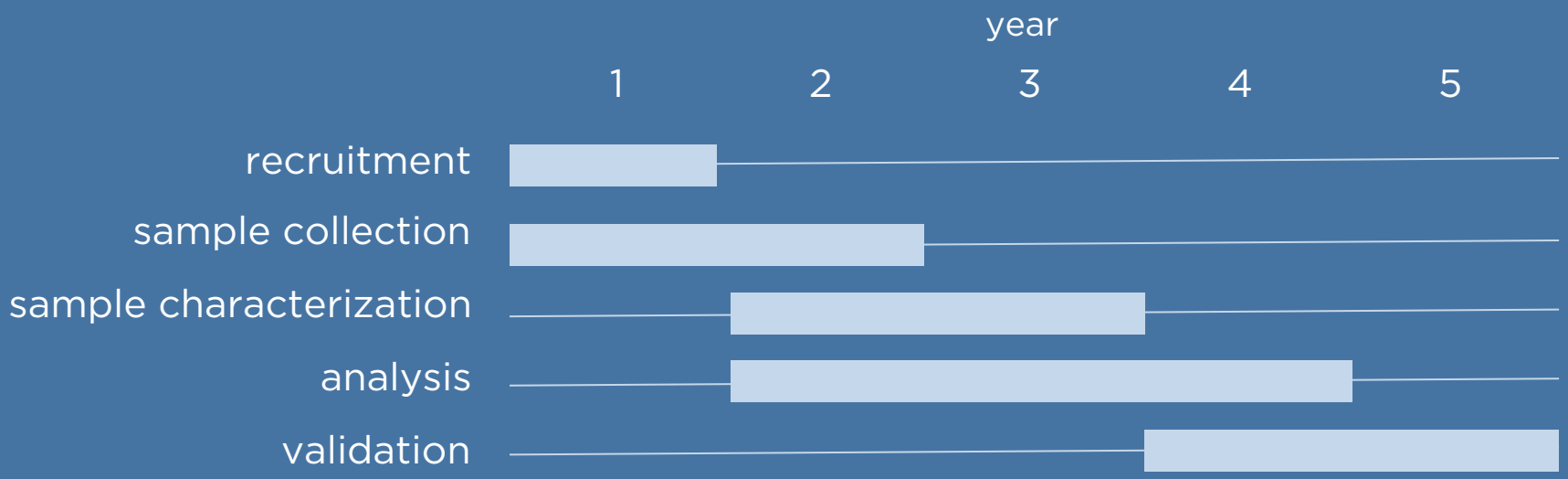
SKIN FIBROBLAST REFERENCE												DONORS (BONE MARROW)
30	○●	30	12	12	FACS			12	12	12		10
30	○	30	(12)	(12)	WHOLE GENOME SHOTGUN (SAMPLES, 8 LANES EACH)			12	(12)	(12)		10
30	●	30	(12)	(12)				12	(12)	(12)		10
	○	30	(12)	(12)	WHOLE TRANSCRIPTOME SHOTGUN (SAMPLES, 0.5 LANES EACH)			12	(12)	(12)		10
	●	30	(12)	(12)				12	(12)	(12)		10
					PCR VALIDATION (EVENTS PER SAMPLE)							
			[100]	[100]	100	100	100	100	[100]	[100]	100	100

( ) - IF SUFFICIENT MATERIAL FOR SEQUENCING

[ ] - IF INSUFFICIENT MATERIAL FOR SEQUENCING

TIMELINE

5 YEARS



THE EXELIXI

ALEX CHANG
RODRIGO GOYA
MARTIN JADERSTEN
MARTIN KRZYWINSKI
EMILIA LIM
MARIA MENDEZ-LAGO
LISA RAEBURN

PI SUPPORT

STEVEN JONES
ROBERT HOLT

BUDGET AND LOGISTICS

ARMELLE TROUSSARD
YJ ZHAO