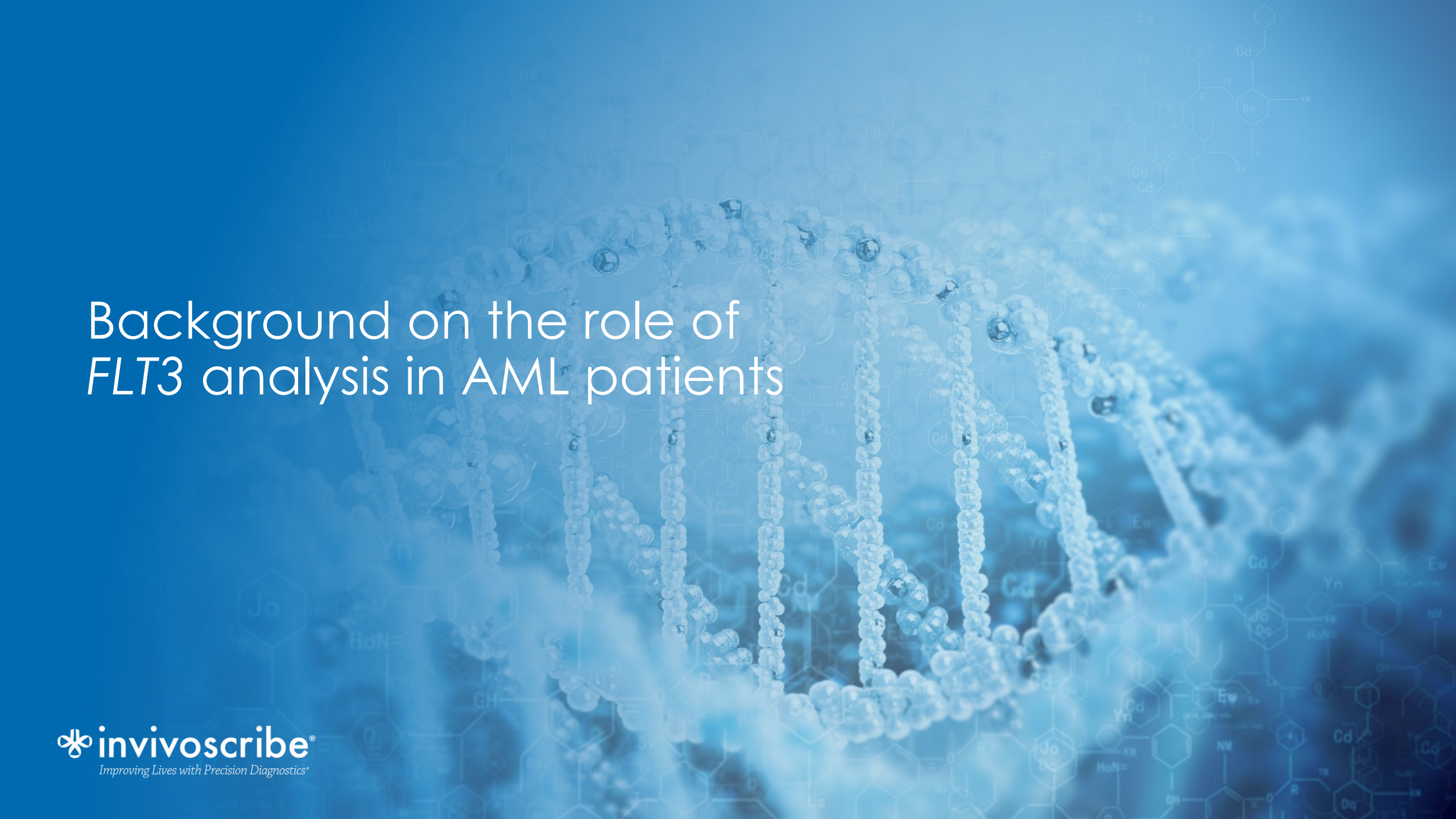
Background on the role of *FLT3* analysis in AML patients



invivoscribe®
Improving Lives with Precision Diagnostics®

FLT3 ITD and TKD Mutations

FLT3-ITD is a common driver mutation in about 1/3 of AML patients and presents with a high leukemic burden and confers a poor prognosis

- Approximately 25% of all AML cases show a *FLT3* ITD mutation
- About 7-10% of all AML cases have a *FLT3* TKD mutation
- Very important markers for risk stratification and for guiding treatment decisions

Daver et. al, "Targeting FLT3 mutations in AML: review of current knowledge and Evidence", Leukemia (2019) 33:299–312

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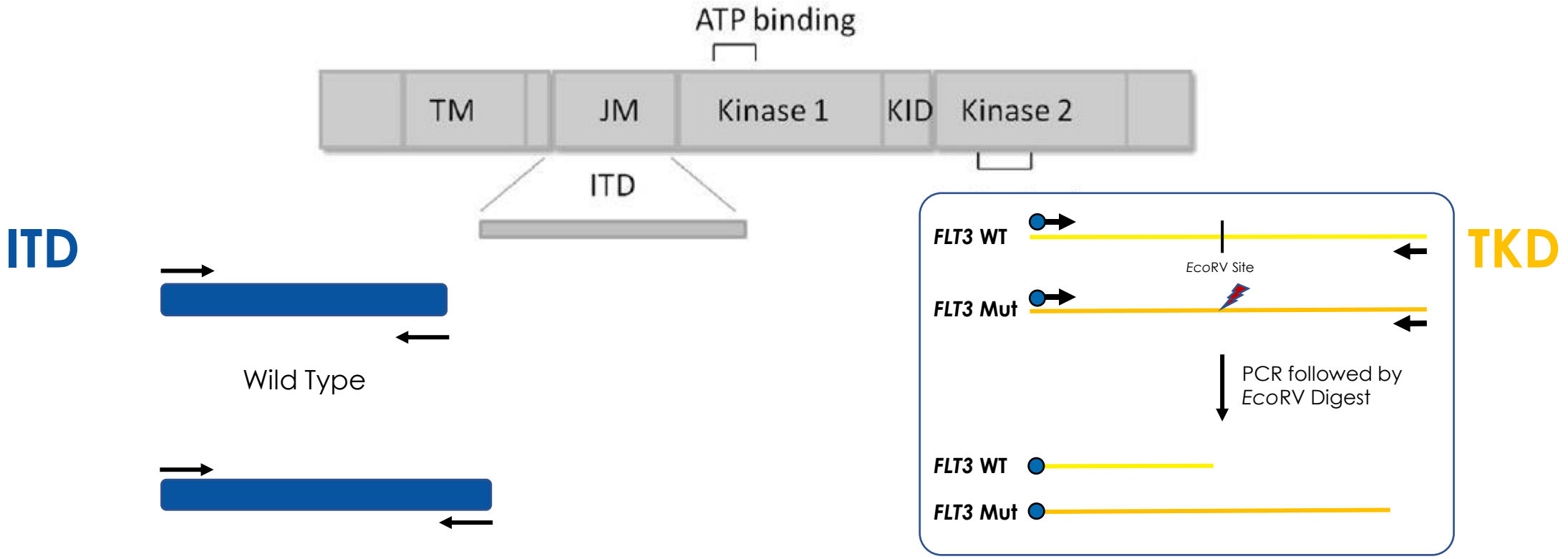
FLT3 ITD and TKD Mutations

Overview of the *FLT3* Gene



Adapted from: Liu et al. Tzu Chi Medical Journal 27 (2015) 18e24

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Prognostic Value of *FLT3* in AML

Common characteristics:

Mutation	Characteristic
FLT3 ITD	Most achieve remission with conventional induction chemotherapy, but they have a pronounced tendency to relapse quickly, and do not live as long compared to AML patients of a similar age lacking such a mutation.
FLT3 TKD	The *FLT3*-TKD mutations are less common and they are also clinically actionable.

Tyrosine Kinase Inhibitors are approved for *FLT3* ITD and/or *FLT3* TKD mutations.

Blood. 2002;99(12):4326-4335
Blood. 2002;100(13):4372-4380
N Engl J Med. 1999;341(14):1051-1062
Nature. 2012;485(7397):260-263

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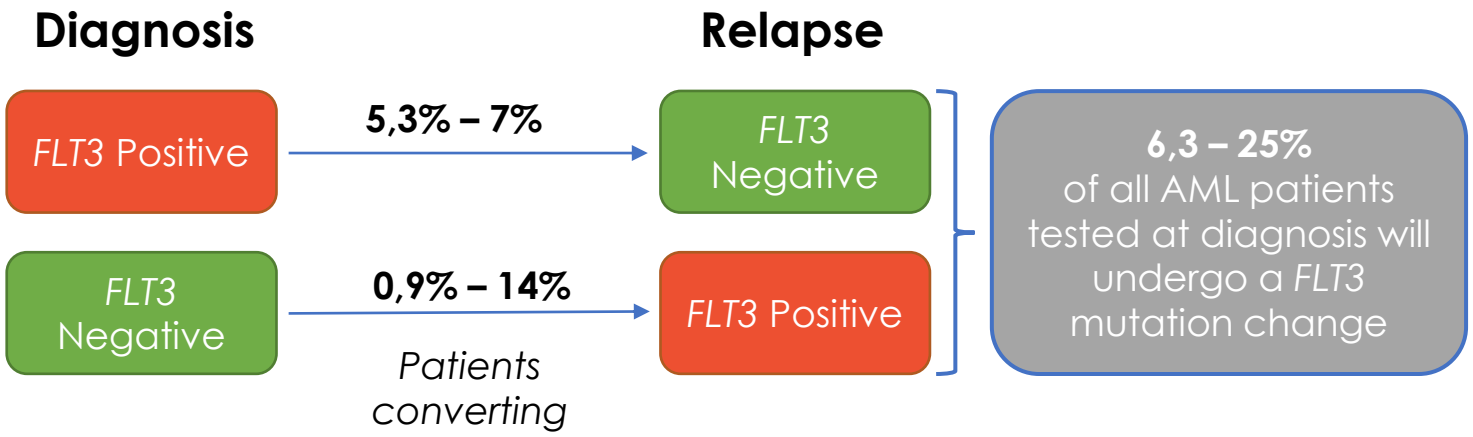
Prognostic Value of *FLT3* in AML

- *FLT3* mutations are unstable and can be gained or lost during disease progression



- **Testing** for *FLT3* mutations **at multiple time points** is important as the status can change from the initial diagnosis to the relapse and refractory states of the disease

Cloos et al. DOI: 10.1038/sj.leu.2404246
Palmisano et al. DOI: 10.3324/haematol.11202
Warren et al. DOI: 10.1038/modpathol.2012.88; published online 8 June 2012

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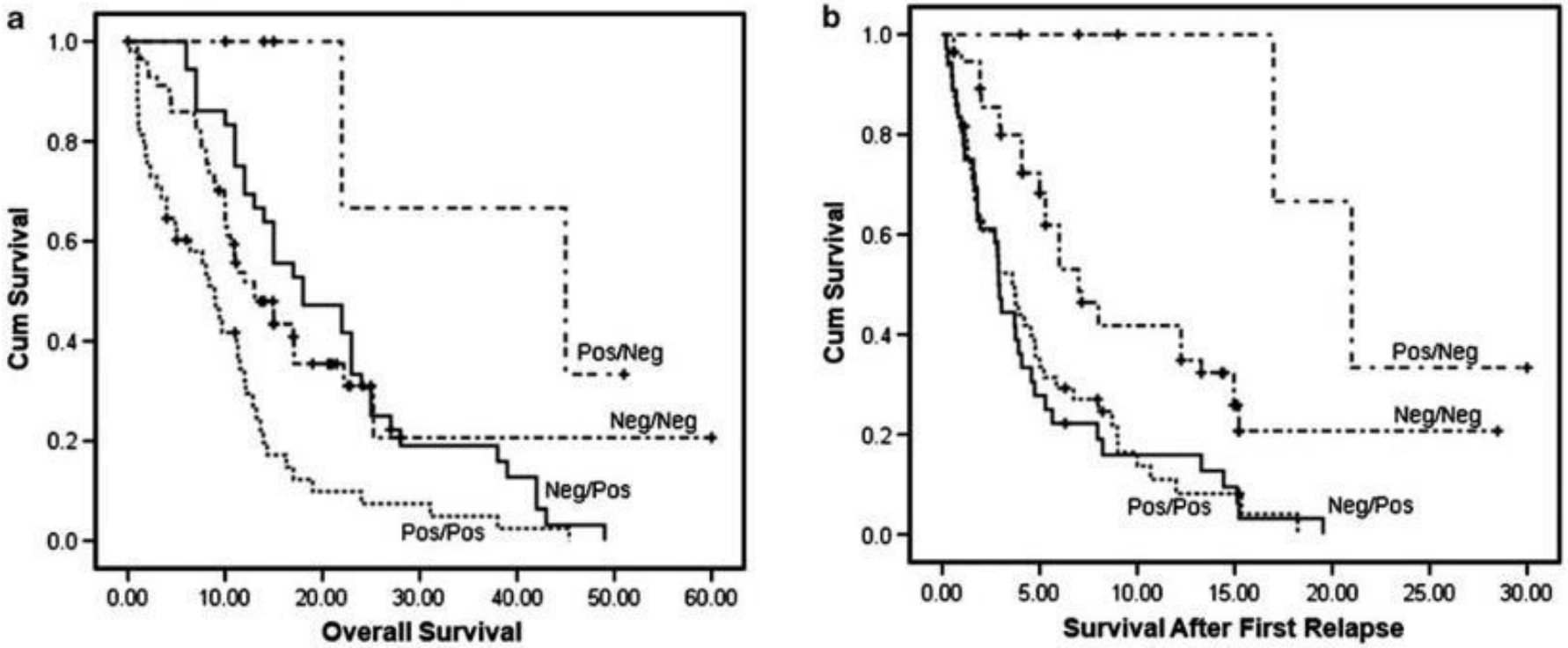
Prognostic Value of *FLT3* in AML

Changes in *FLT3* mutation status is an important prognostic factor

- Study of 3355 patients



Warren et al. DOI: 10.1038/modpathol.2012.88; published online 8 June 2012

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International Guidelines

FLT3 testing is recommended as part of international AML guidelines

ELN European LeukemiaNet

NCCN National Comprehensive Cancer Network®

(doi: 10.6004/jnccn.2019.0028)

Risk category*	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}†
	Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high}†
	Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}† (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*‡
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214*
	t(v;11q23.3); *KMT2A* rearranged
	t(9;22)(q34.1;q11.2); *BCR-ABL1*
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)*
	−5 or del(5q); −7; −17/abn(17p)
	Complex karyotype,§ monosomal karyotype‖
	Wild-type *NPM1* and *FLT3*-ITD^{high}†
	Mutated *RUNX1*¶
	Mutated *ASXL1*¶
	Mutated *TP53*#

Blood. 2017;129(4):424-447

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Determination of high/low classification for ITD SR has not been validated for Invivoscribe products.

The Role of Allelic Ratio in AML

Different opinions in the clinical community

National Comprehensive Cancer Network®

Low allelic ratio (AR) (<0.5); High AR(≥0.5)

> "Regardless of *FLT3* AR, patients should be considered for bone marrow transplant"

> "Though recent studies indicate that AML with NPM1 mutation and *FLT3*-ITD low AR may have a more favorable prognosis and patients should not routinely be assigned to allogeneic HCT"

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

NCCN Guidelines / Acute Myeloid Leukemia / Version 2020.3

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ELN Studies Cited for AR Criteria

Publication (Trial)	Date	First Author	ITD High Low Cutoff
Blood (UK MRC AML)	2008	Gale	< 25% Low 25% – 50% Int. >50% High
Blood (CETLAM)	2013	Pratcorona	High > 0.5
Blood (UK MRC AML)	2014	Linch	5%-25% Low 25%-50% Int. >50% High
Blood (AMLSG)	2014	Schlenk	High > 0.51
NEJM (Ratify)	2017	Stone	High > 0.7

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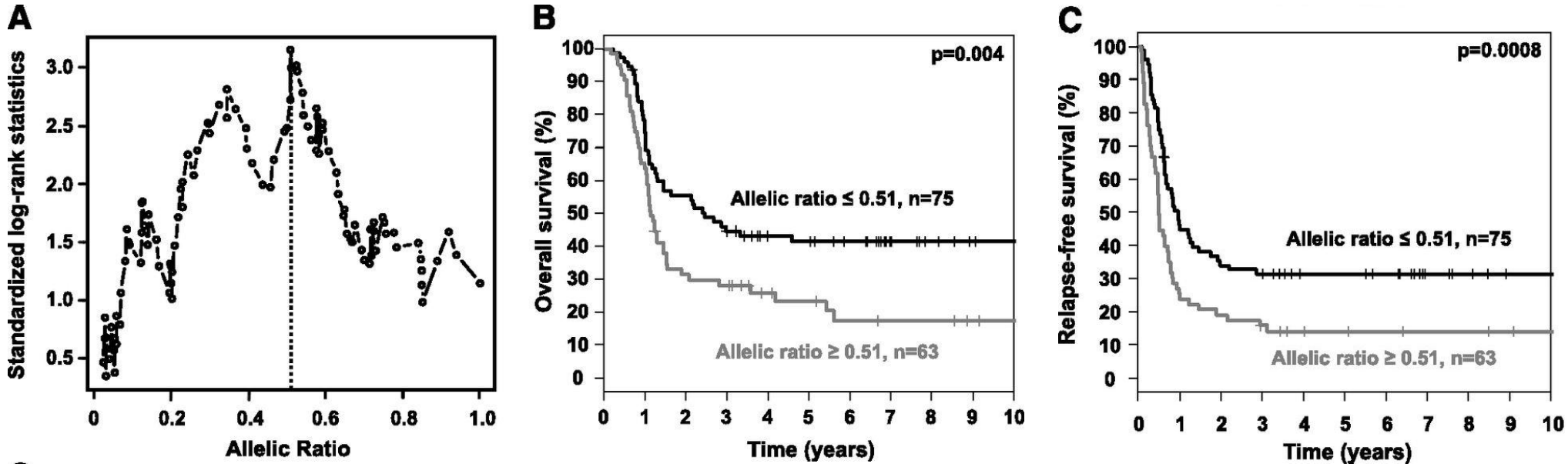
Impact of *FLT3* AR in OS

Differential impact of allelic ratio and insertion site in *FLT3*-ITD-positive AML with respect to allogeneic transplantation



Richard F. Schlenk, Sabine Kayser, Lars Bullinger, Guido Kobbe, Jochen Casper, Mark Ringhoffer, Gerhard Held, Peter Brossart, Michael Lübbert, Helmut R. Salih, Thomas Kindler, Heinz A. Horst, Gerald Wulf, David Nachbaur, Katharina Götze, Alexander Lamparter, Peter Paschka, Verena I. Gaidzik, Veronica Teleanu, Daniela Späth, Axel Benner, Jürgen Krauter, Arnold Ganser, Hartmut Döhner and Konstanze Döhner for the German-Austrian AML Study Group. https://doi.org/10.1182/blood-2014-05-578070

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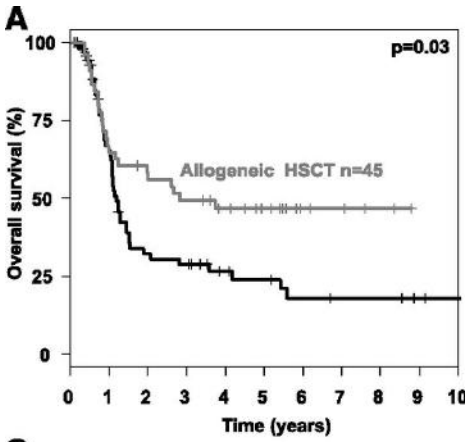
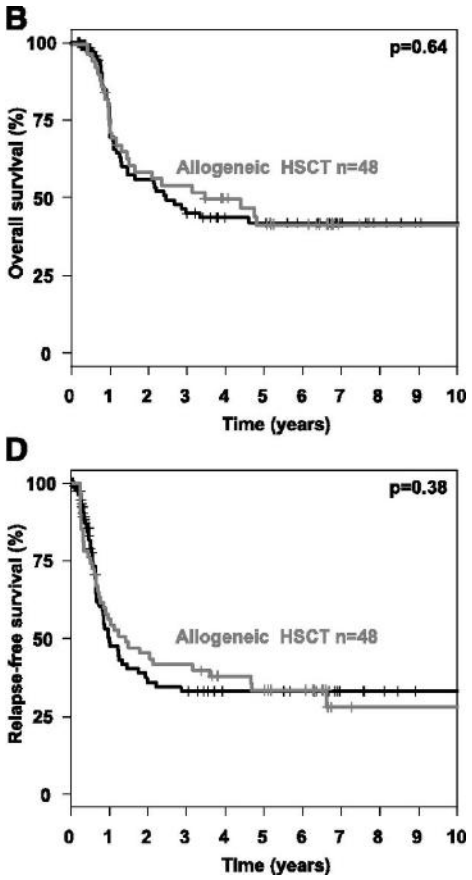
Impact of *FLT3* AR in OS

AR in *FLT3*-ITD–positive AMLs may be useful as a predictive marker indicating whether an alloHSCT in first CR is beneficial in terms of overall outcome.

High ITD



Low ITD



https://doi.org/10.1182/blood-2014-05-578070

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Prevalence of AML and Mutations

NATIONAL CANCER INSTITUTE
Surveillance, Epidemiology, and End Results Program

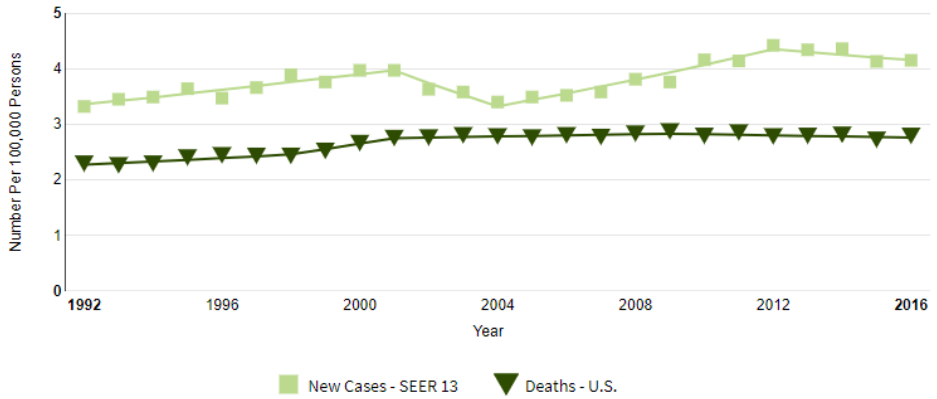
21,450 new cases x (0.30 NPM1mut) x (0.40 FLT3 ITD) = 2,574 new cases/year

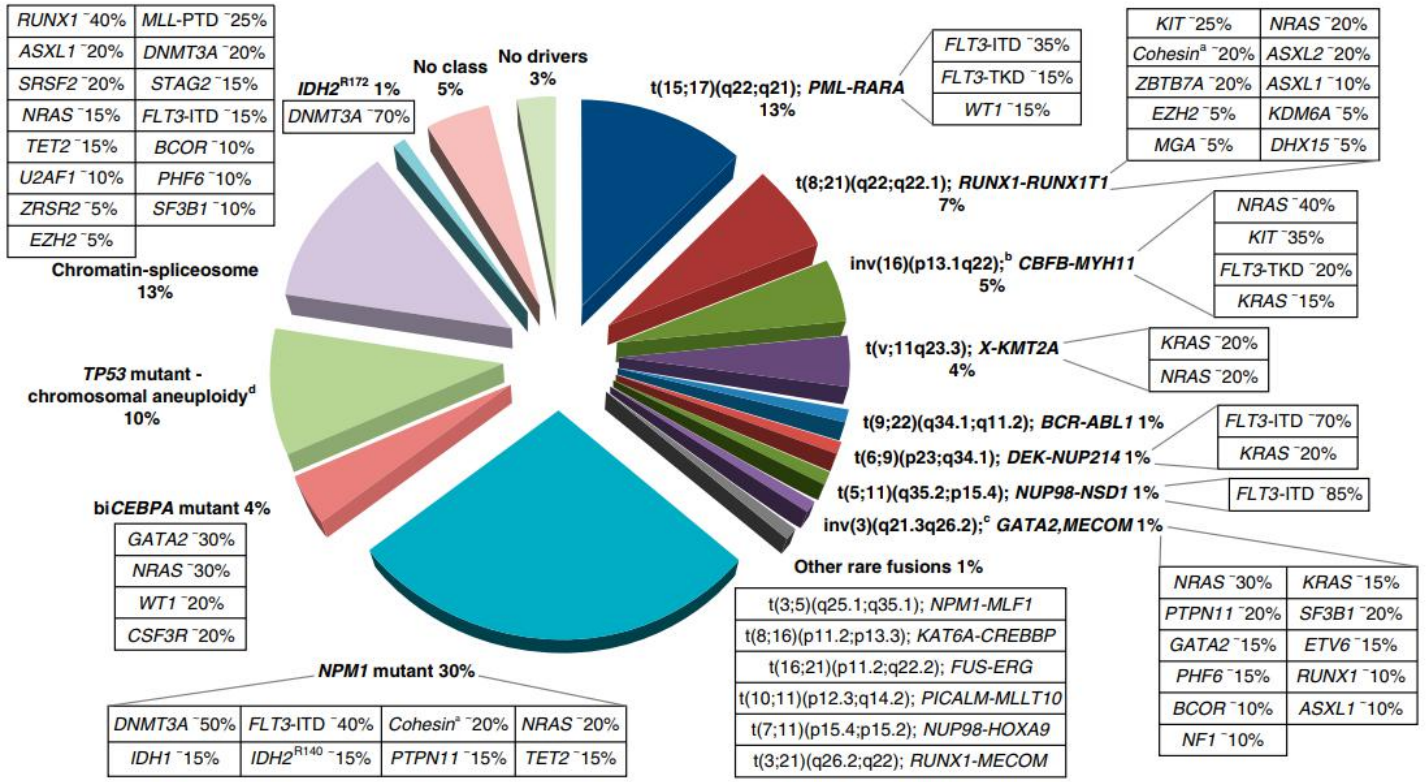
At a Glance

Estimated New Cases in 2019	21,450
% of All New Cancer Cases	1.2%

Estimated Deaths in 2019	10,920
% of All Cancer Deaths	1.8%

Percent Surviving 5 Years
28.3%
2009-2015





Blood. 2017; 129(4):424-447

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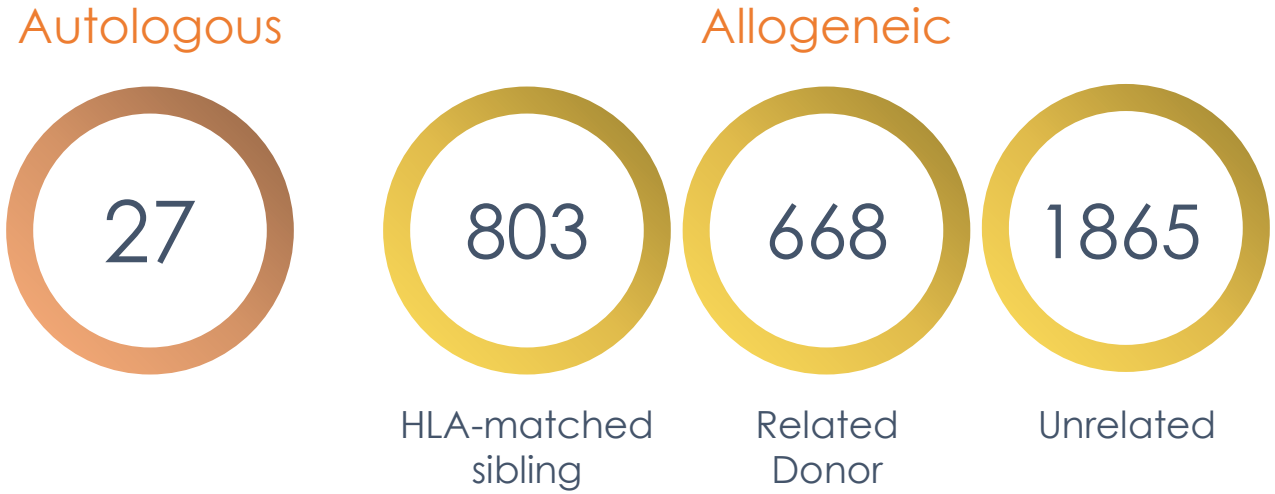
Number of Transplants in AML

US Transplant Data by Center Report – AML 2017

HRSA
Health Resources & Services Administration



Total: 3363

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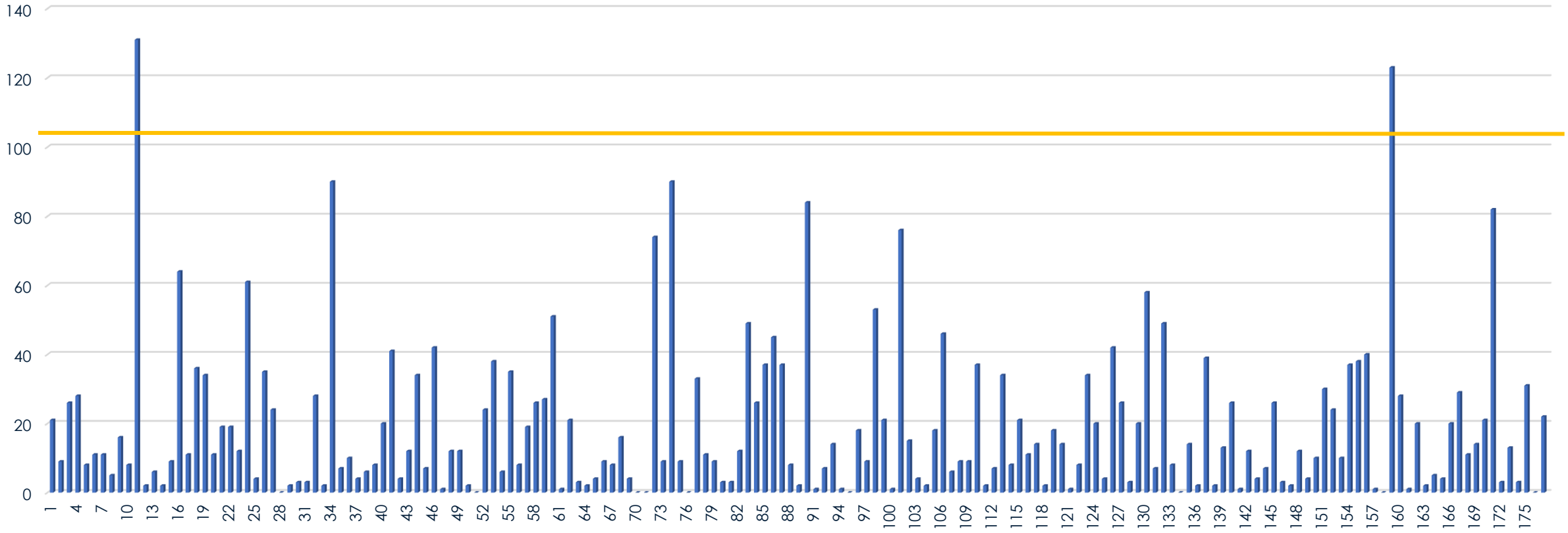
Experience matters

HRSA
Health Resources & Services Administration

Transplants by Center 2017



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