ゲノムバイオマーカーに基づいたがん治療選択

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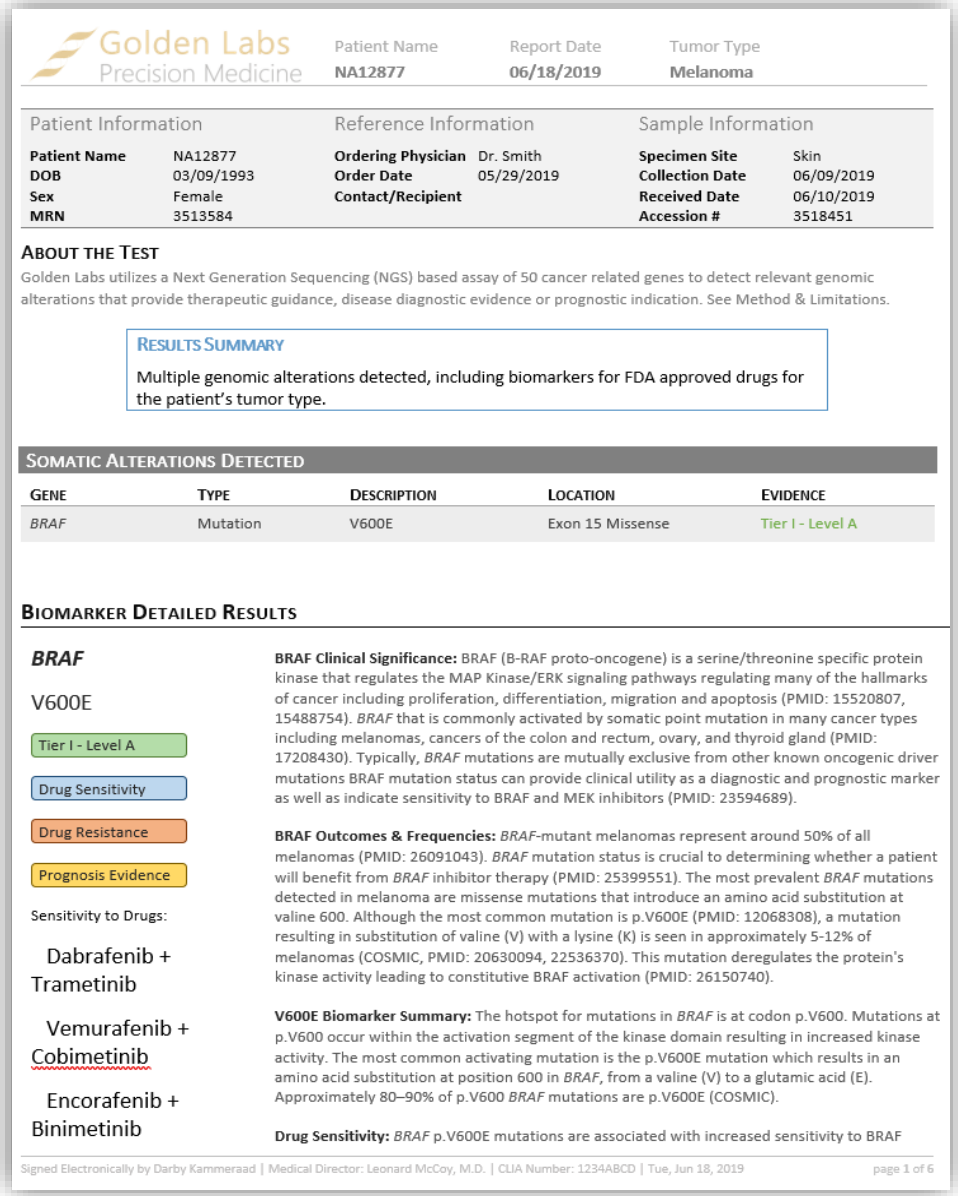
はじめに

- 遺伝子パネル検査によるがんゲノム医療では、検出された遺伝子変異などのゲノムバイオマーカーについて、推奨される治療薬などの臨床的意義を確認する必要がある

  バイオマーカー例
  - HER2+: High levels of HER2 receptor protein
  - MSI-H: Microsatellite instability-high
  - $BRAF^{V600E}$: Activating mutation V600E
  - $ERBB2^{Amp}$: Amplification of ERBB2
  - BCR-ABL1: Activation of ABL1 with BCR fusion
  - $TP53^{WT}$: No significant alterations of critical TSG

- Golden Helix社VarSeq®の有償アドオンであるVSClinical AMPでは、体細胞バリアントの腫瘍原性の評価に加え、バイオマーカーとしてのエビデンスレベルや治療薬、臨床試験情報の確認を行い、レポートにまとめて出力することが可能



Golden Labs Precision Medicine

Patient Name	Report Date	Tumor Type
NA12877	06/18/2019	Melanoma

Patient Information

Patient Name	NA12877
DOB	03/09/1993
Sex	Female
MRN	3513584

Reference Information

Ordering Physician	Dr. Smith
Order Date	05/29/2019
Contact/Recipient	

Sample Information

Specimen Site	Skin
Collection Date	06/09/2019
Received Date	06/10/2019
Accession #	3518451

About the Test

Golden Labs utilizes a Next Generation Sequencing (NGS) based assay of 50 cancer related genes to detect relevant genomic alterations that provide therapeutic guidance, disease diagnostic evidence or prognostic indication. See Method & Limitations.

Results Summary

Multiple genomic alterations detected, including biomarkers for FDA approved drugs for the patient's tumor type.

Somatic Alterations Detected

Gene	Type	Description	Location	Evidence
BRAF	Mutation	V600E	Exon 15 Missense	Tier I - Level A

Biomarker Detailed Results

BRAF

V600E

Tier I - Level A

Drug Sensitivity

Drug Resistance

Prognosis Evidence

Sensitivity to Drugs:

Dabrafenib + Trametinib

Vemurafenib + Cobimetinib

Encorafenib + Binimetinib

BRAF Clinical Significance: BRAF (B-RAF proto-oncogene) is a serine/threonine specific protein kinase that regulates the MAP Kinase/ERK signaling pathways regulating many of the hallmarks of cancer including proliferation, differentiation, migration and apoptosis (PMID: 15520807, 15488754). *BRAF* that is commonly activated by somatic point mutation in many cancer types including melanomas, cancers of the colon and rectum, ovary, and thyroid gland (PMID: 17208430). Typically, *BRAF* mutations are mutually exclusive from other known oncogenic driver mutations BRAF mutation status can provide clinical utility as a diagnostic and prognostic marker as well as indicate sensitivity to BRAF and MEK inhibitors (PMID: 23594689).

BRAF Outcomes & Frequencies: *BRAF*-mutant melanomas represent around 50% of all melanomas (PMID: 26091043). *BRAF* mutation status is crucial to determining whether a patient will benefit from *BRAF* inhibitor therapy (PMID: 25399551). The most prevalent *BRAF* mutations detected in melanoma are missense mutations that introduce an amino acid substitution at valine 600. Although the most common mutation is p.V600E (PMID: 12068308), a mutation resulting in substitution of valine (V) with a lysine (K) is seen in approximately 5-12% of melanomas (COSMIC, PMID: 20630094, 22536370). This mutation deregulates the protein's kinase activity leading to constitutive BRAF activation (PMID: 26150740).

V600E Biomarker Summary: The hotspot for mutations in *BRAF* is at codon p.V600. Mutations at p.V600 occur within the activation segment of the kinase domain resulting in increased kinase activity. The most common activating mutation is the p.V600E mutation which results in an amino acid substitution at position 600 in *BRAF*, from a valine (V) to a glutamic acid (E). Approximately 80–90% of p.V600 *BRAF* mutations are p.V600E (COSMIC).

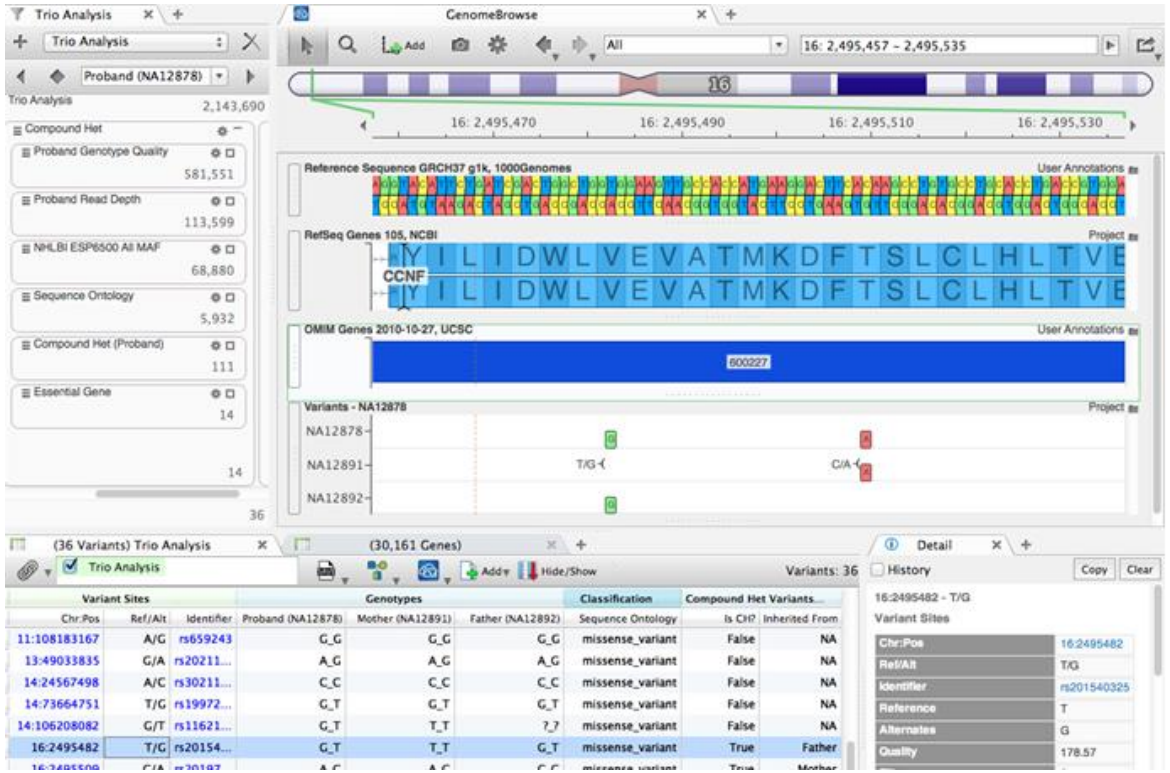
Drug Sensitivity: *BRAF* p.V600E mutations are associated with increased sensitivity to BRAF

Signed Electronically by Darby Kammeraad | Medical Director: Leonard McCoy, M.D. | CLIA Number: 1234ABCD | Tue, Jun 18, 2019 page 1 of 6

VarSeq®

Annotation

Variant Filtering



Data Analysis

Genome Browser

■ 様々なデータリソースを用いて、変異データ（VCFファイル）へアノテーション付けを実行

- RefSeq Genes
- dbSNP
- ClinVar
- OMIM
- COSMIC
- CIVic
- ICGC / TCGA
- Orphanet
- BRCA Exchange
- MSK Impact
- PMKB
- dbNSFP
- REVEL
- CADD
- 1000 Genomes
- NHLBI 6500 Exomes
- ExAC Variant
- gnomAD Exomes/Genomes
- GenomeAsia 100K
- 各種遺伝子パネルのターゲットデータ

…など

■ アノテーション付けされた変異データより、任意の検索条件でデータのフィルタリングを行うワークフローを作成

■ カバレッジ計算やトリオ解析、表現型情報に基づく遺伝子ランキングなどの解析アルゴリズムを搭載

■ ゲノムブラウザーを搭載し、BAMファイルデータや各種アノテーションデータをグラフ表示

■ 有償アドオンによる機能拡張で、CNVコールやレポート作成、パイプライン機能などが利用可能

VSClinical

VSClinical (ACMG)

- メンデル遺伝病における生殖細胞系列バリアントを、ACMGガイドラインの33種類の評価基準に基づいて分類し、病原性（Pathogenic）や良性（Benign）の判定を行う
- ガイドラインのうち18種類の評価基準については、バリアントのアレル頻度、機能予測、臨床情報データベースなどを用いて、自動分類を実行
- 専用の分類用ツールを実行することで、VCFファイルに含まれる全バリアントに対して一括で評価を行い、評価結果に基づきバリアントのフィルタリングが可能

VSClinical (AMP)

- がんにおける体細胞バリアントの臨床的意義の評価に使用
- 体細胞バリアントの腫瘍原性（Oncogenicity）をスコアで評価
- 各種バイオマーカー（Small Variant, CNV, 融合遺伝子など）に対して、AMPガイドラインのEvidence tierレベルで分類し、治療薬や臨床試験情報を含めたレポートを作成
- 主要ながんにおけるバイオマーカー情報などを収録した、専用の知識ベースGolden Helix CancerKBが利用可能

VSClinical AMP Workflow

VCF

BAM

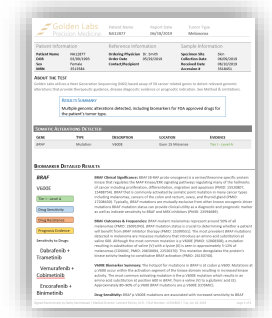
- Variant Filtering
- Sequencing QC

Oncogenicity Classification

Evidence Assessment

Drug & Clinical Trials Review

Report Generation

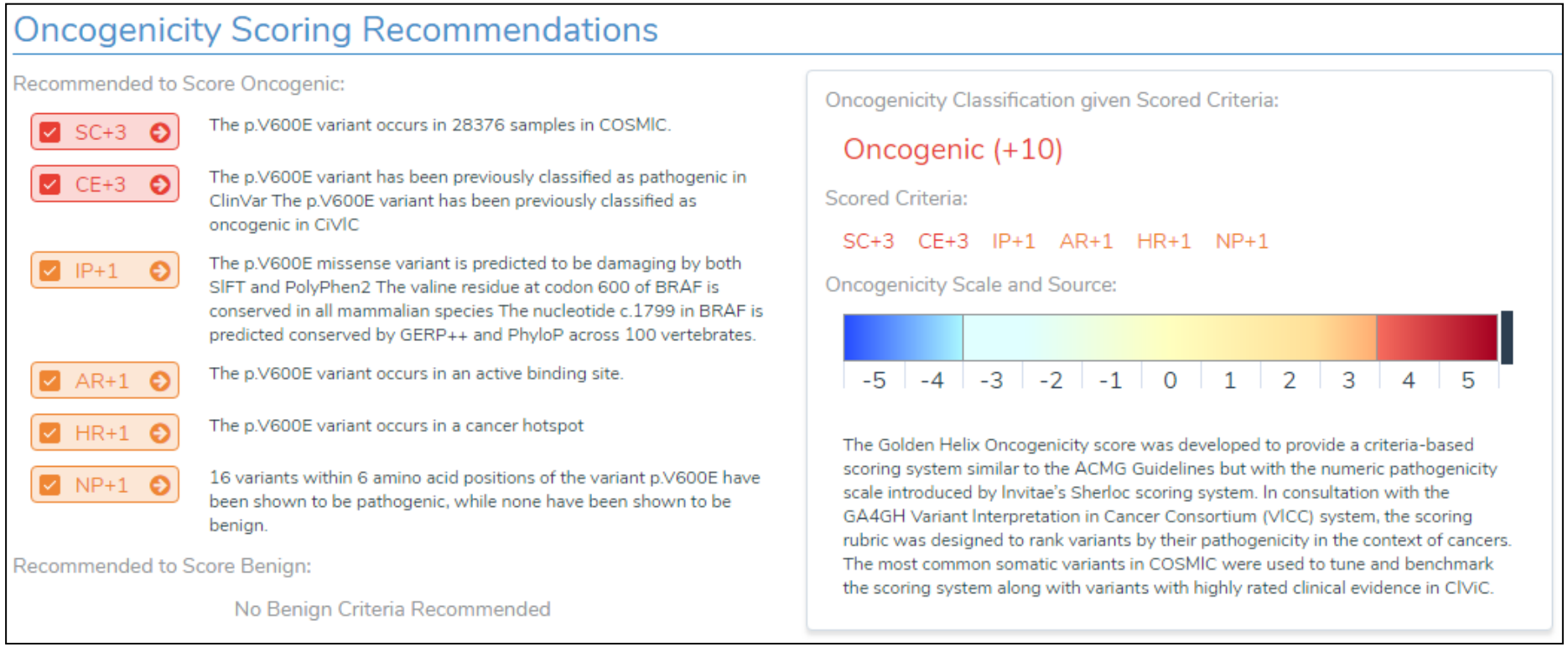


varSEQ®

VSClinical®

Oncogenicity Scoring

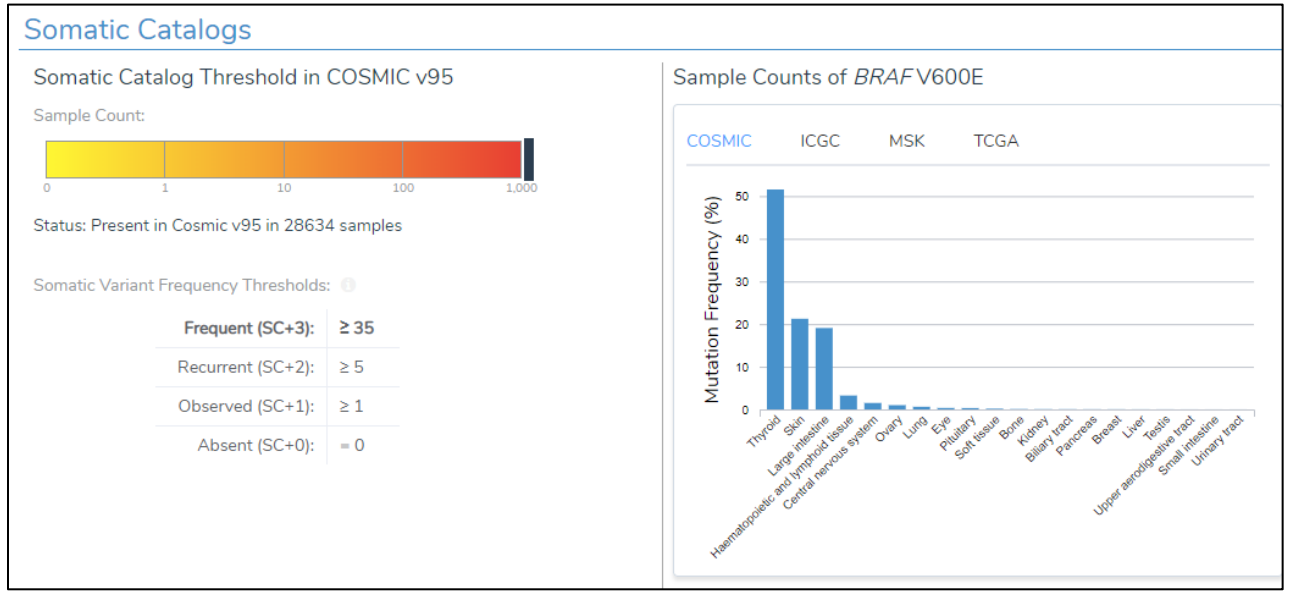
Oncogenicity Classification

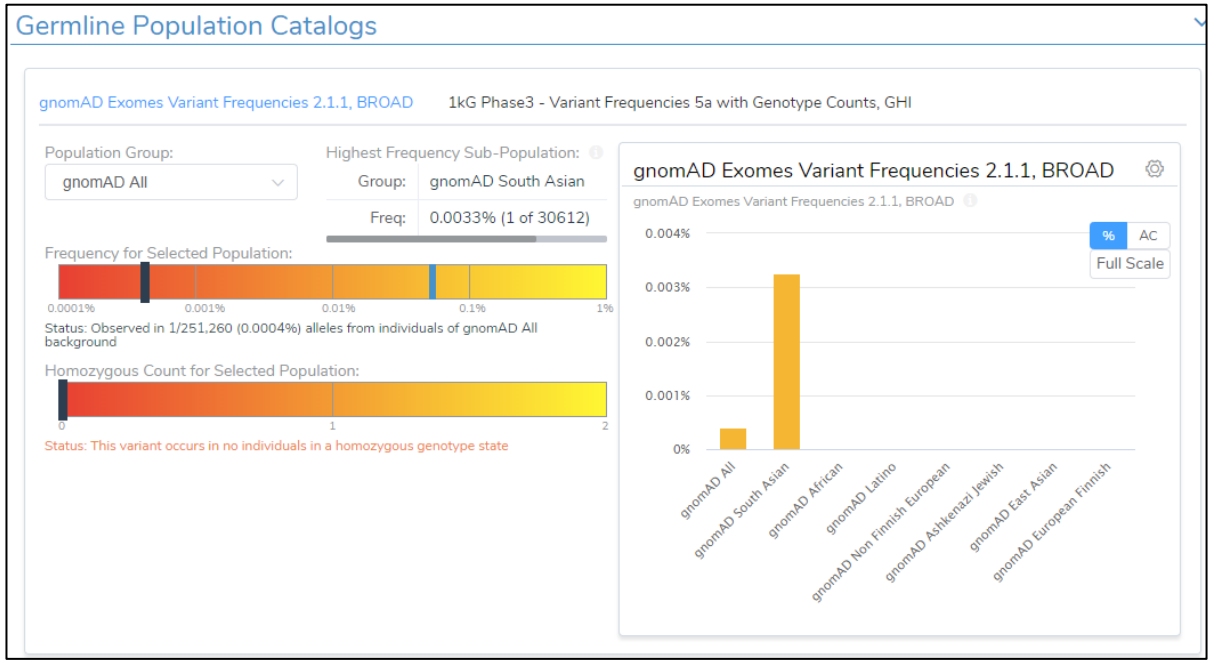


- Golden Helix社独自開発の、体細胞バリアントの腫瘍原性評価アルゴリズム
- 評価結果のスコアに応じて、Oncogenic, Benign, VUSなどに自動で分類される

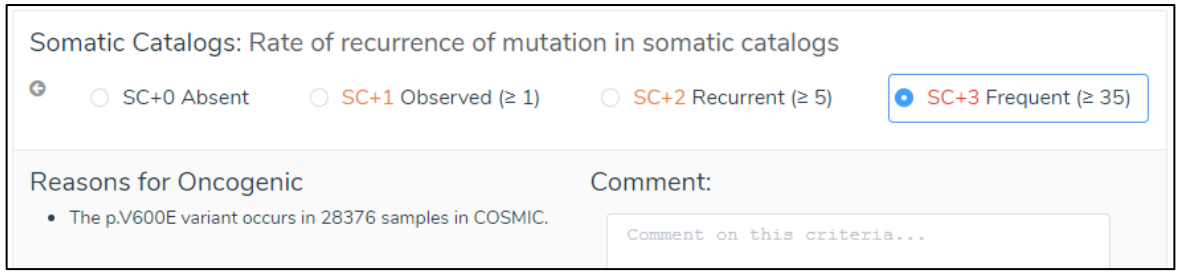
- 評価項目例
  - Germline Population Catalogs
  - In-Silico Functional/Splicing
  - Previous / Clinical Evaluations
  - Somatic Catalogs
  - Domain / Hotspot Analysis
  - Gene Affinity to Variant Type

評価データの確認

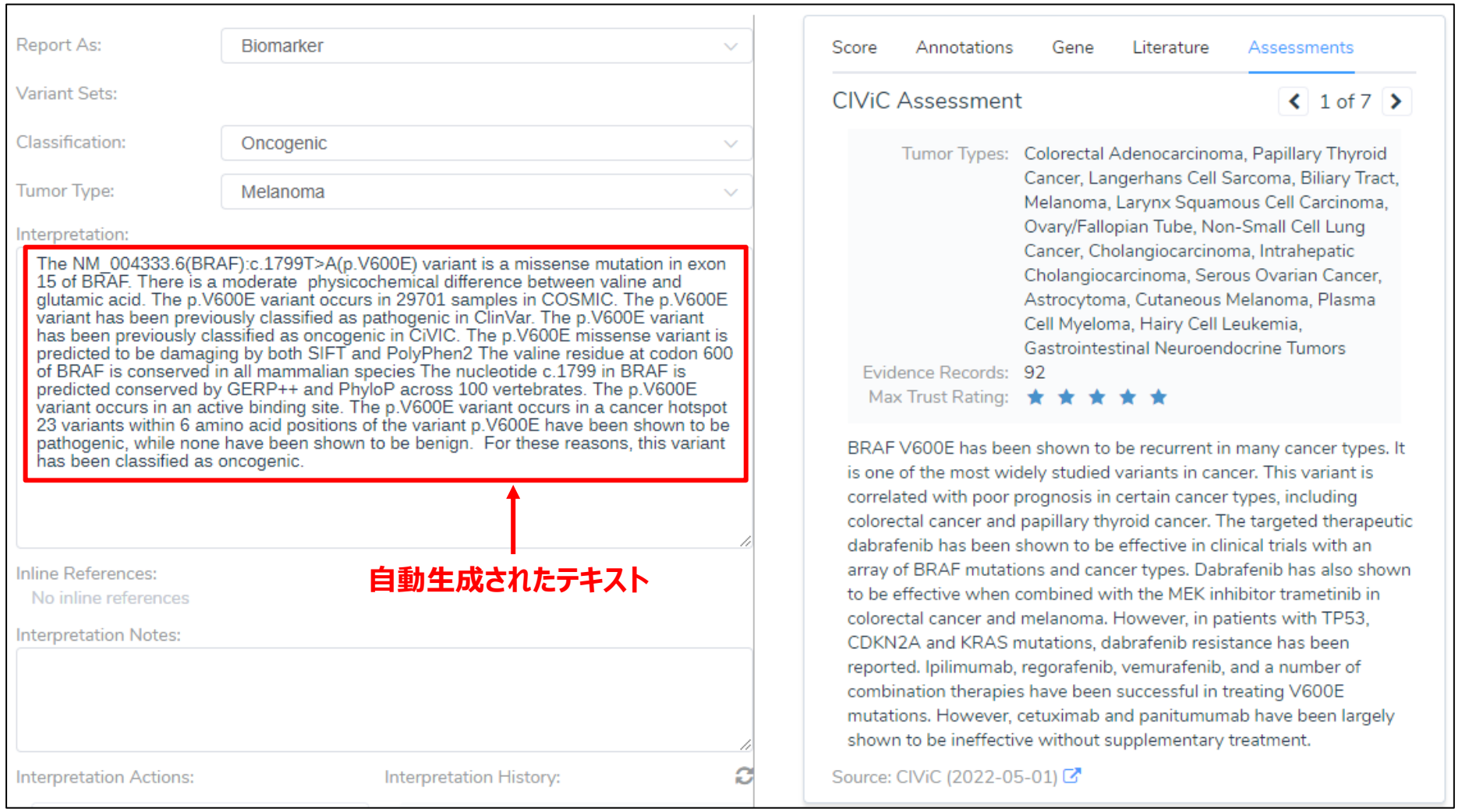




- ダッシュボードで評価項目ごとに関連データを確認
- 項目ごとに評価判定の根拠の確認や、評価スコアを手動で調整することも可能

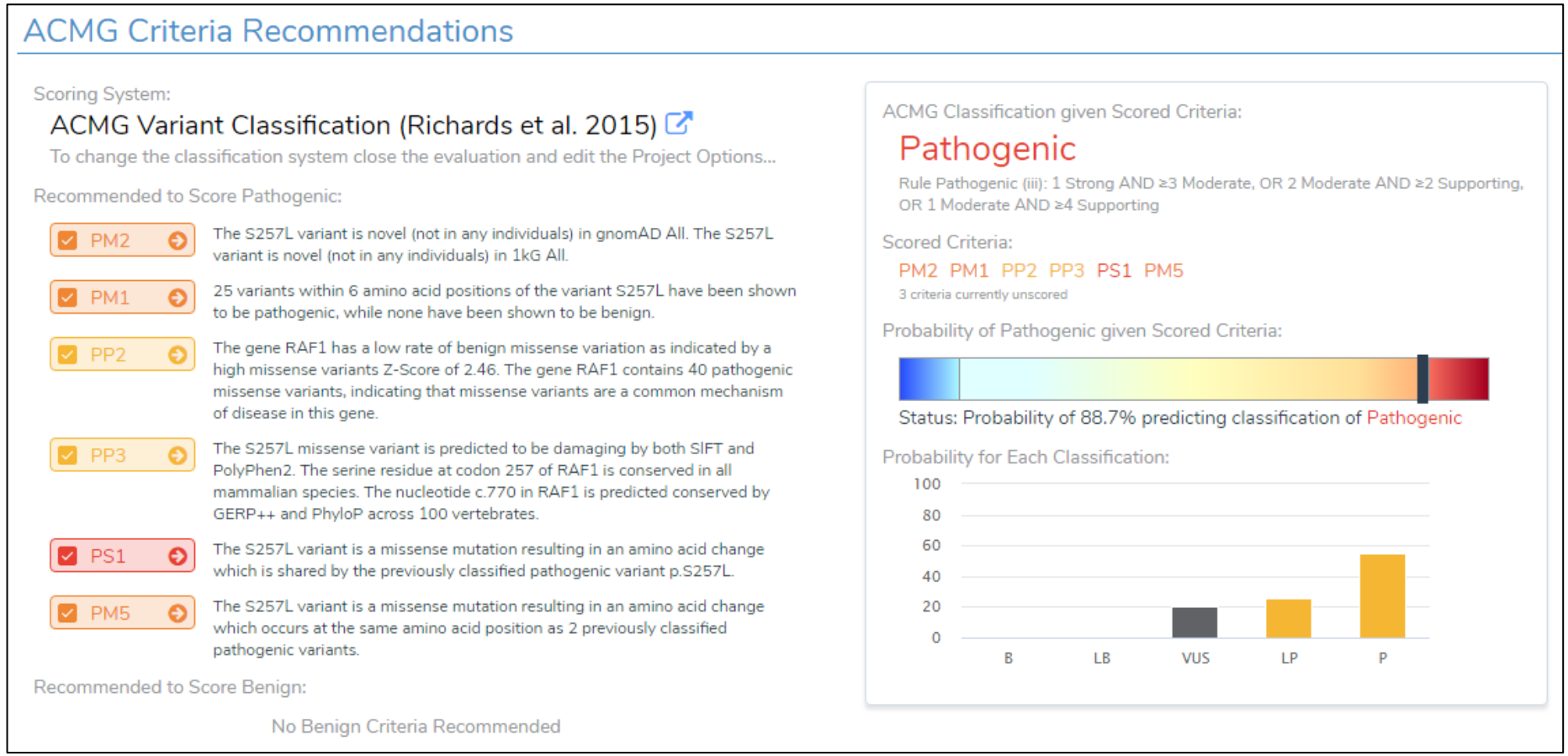


臨床的解釈のレポート用テキスト



- 評価結果に基づき、臨床的解釈のテキストが自動生成される
- 参考文献やデータベースの情報なども、インラインで追加が可能

生殖細胞系列バリアントの評価

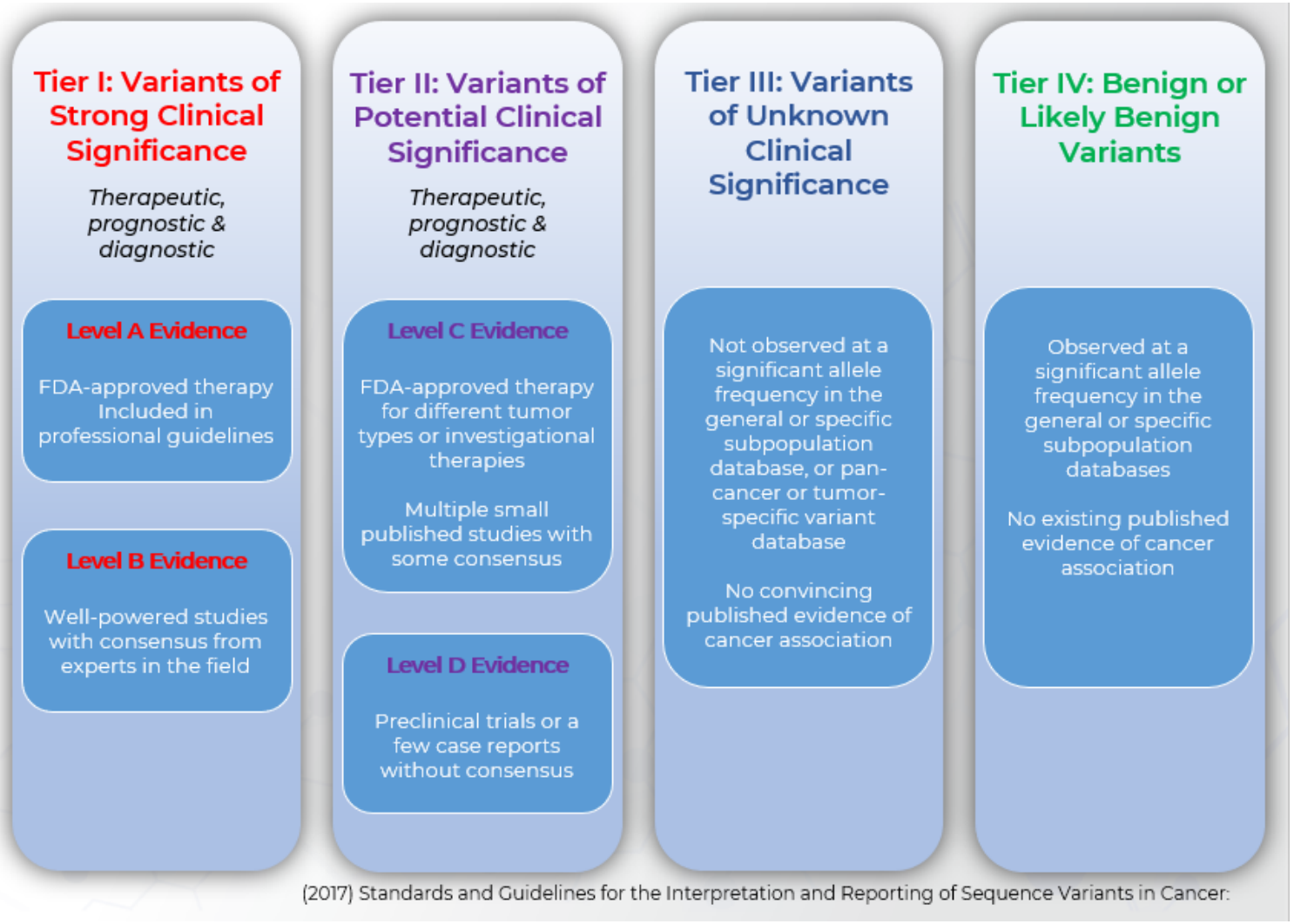


- 生殖細胞系列バリアントの評価には、ACMGガイドラインの評価項目を使用
- Secondary Germlineとしてレポートに追加も可能

AMPガイドライン

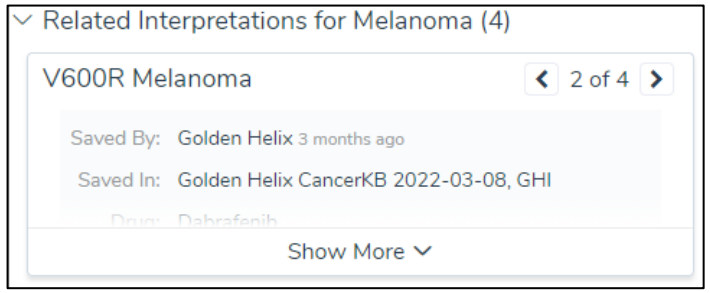
Evidence Assessment

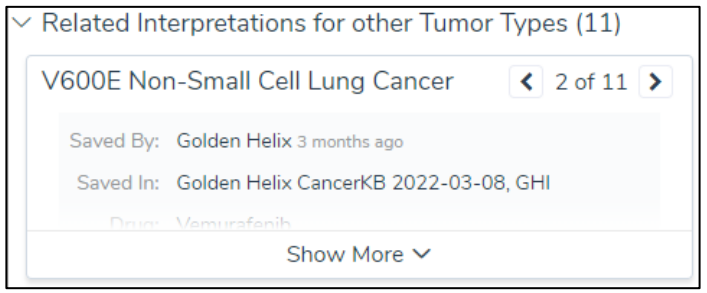
- バイオマーカーに対する、がんの治療方法のアクショナビリティに基づいた分類ガイドライン
- 治療薬の感受性や耐性、さらに予後／診断に関する臨床的な評価を、エビデンスレベルで分類を行う
- 同一バイオマーカーの場合でも、がん種ごとに分類結果は異なる
- VSClinical AMPでは、知識ベースから情報を引き出し、バイオマーカーごとのエビデンスレベルを、ソフトウェアが自動的に分類を行う

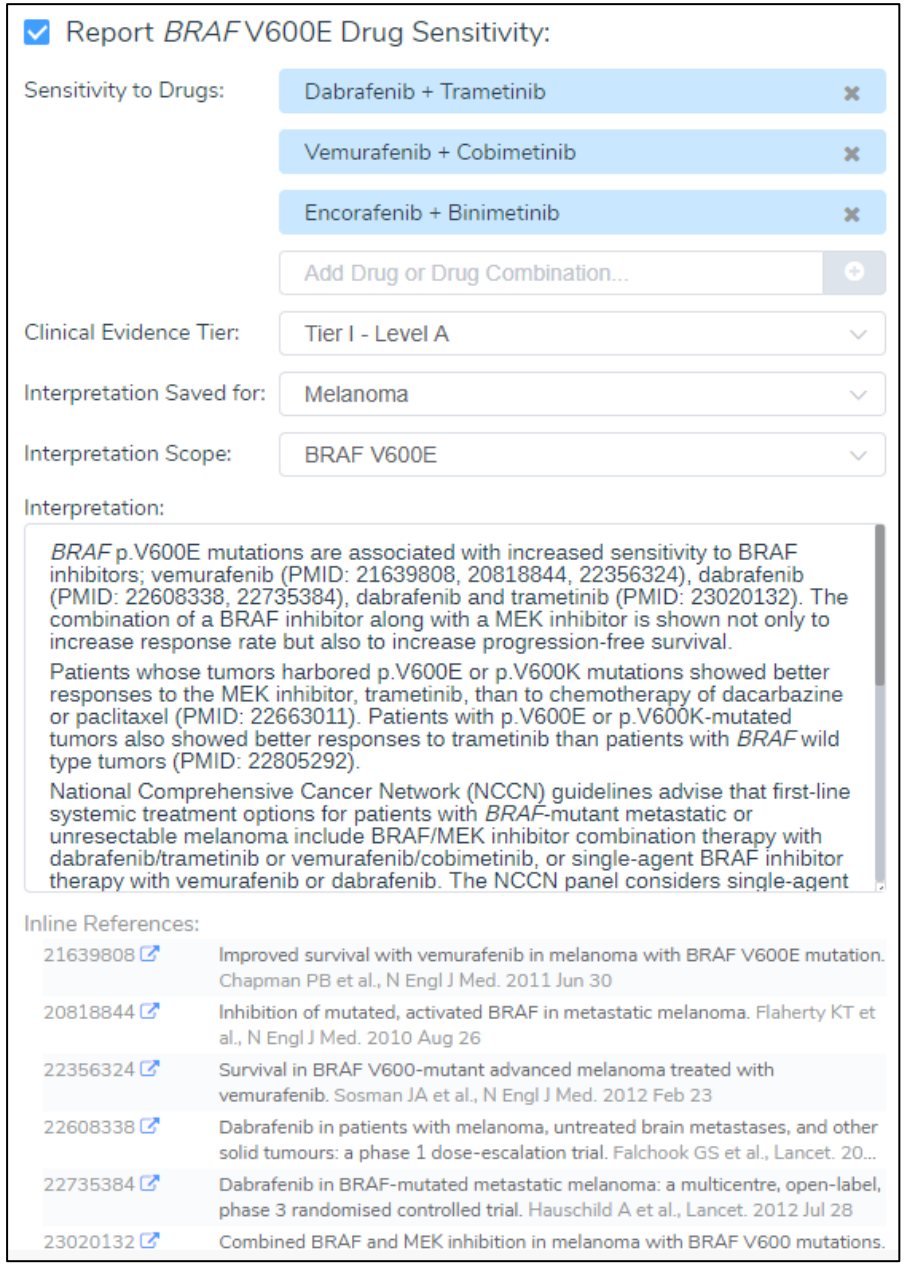


Golden Helix CancerKB

- Golden Helix社の臨床エキスパートがマニュアルキュレーションで作成した、がんゲノム医療向けの知識ベース
- 遺伝子またはバイオマーカーごとに、該当がん種のエビデンスレベル、臨床的解釈や候補治療薬の組み合わせ、参考論文などの情報が収録されている
- 臨床的解釈は7つのセクションに分類されており、目的に応じて使い分けが可能
  - Gene Summary
  - Outcomes & Frequency
  - Biomarker Summary
  - Clinical Evidence
    - ・Drug Sensitivity
    - ・Drug Resistance
    - ・Prognostic
    - ・Diagnostic
- 同一がん種における異なるバイオマーカーや、異なるがん種における同一バイオマーカーなどの情報も確認可能





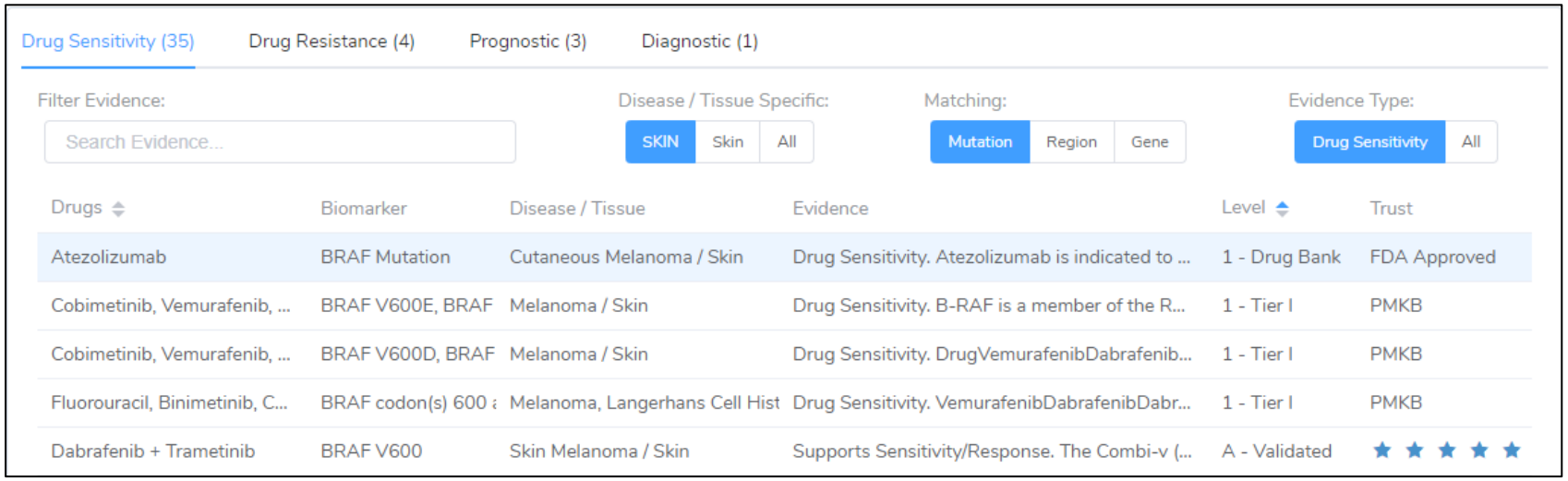


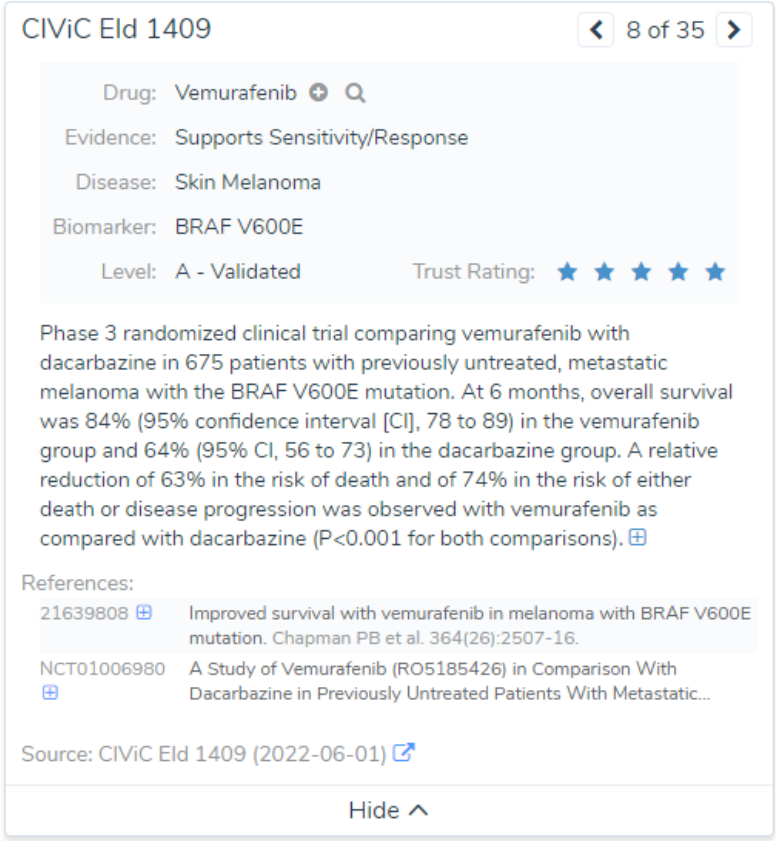
その他の知識ベース

- Golden Helix CancerKB以外に、公共データベースの知識ベースを活用することも可能
  - DrugBank
  - CIViC
  - PMKB (Precision Medicine Knowledgebase)

- Golden Helix CancerKBと同様に、バイオマーカーごとに該当がん種のエビデンスレベル、臨床的解釈や候補治療薬、参考論文などの情報が、研究レコードごとに納められている

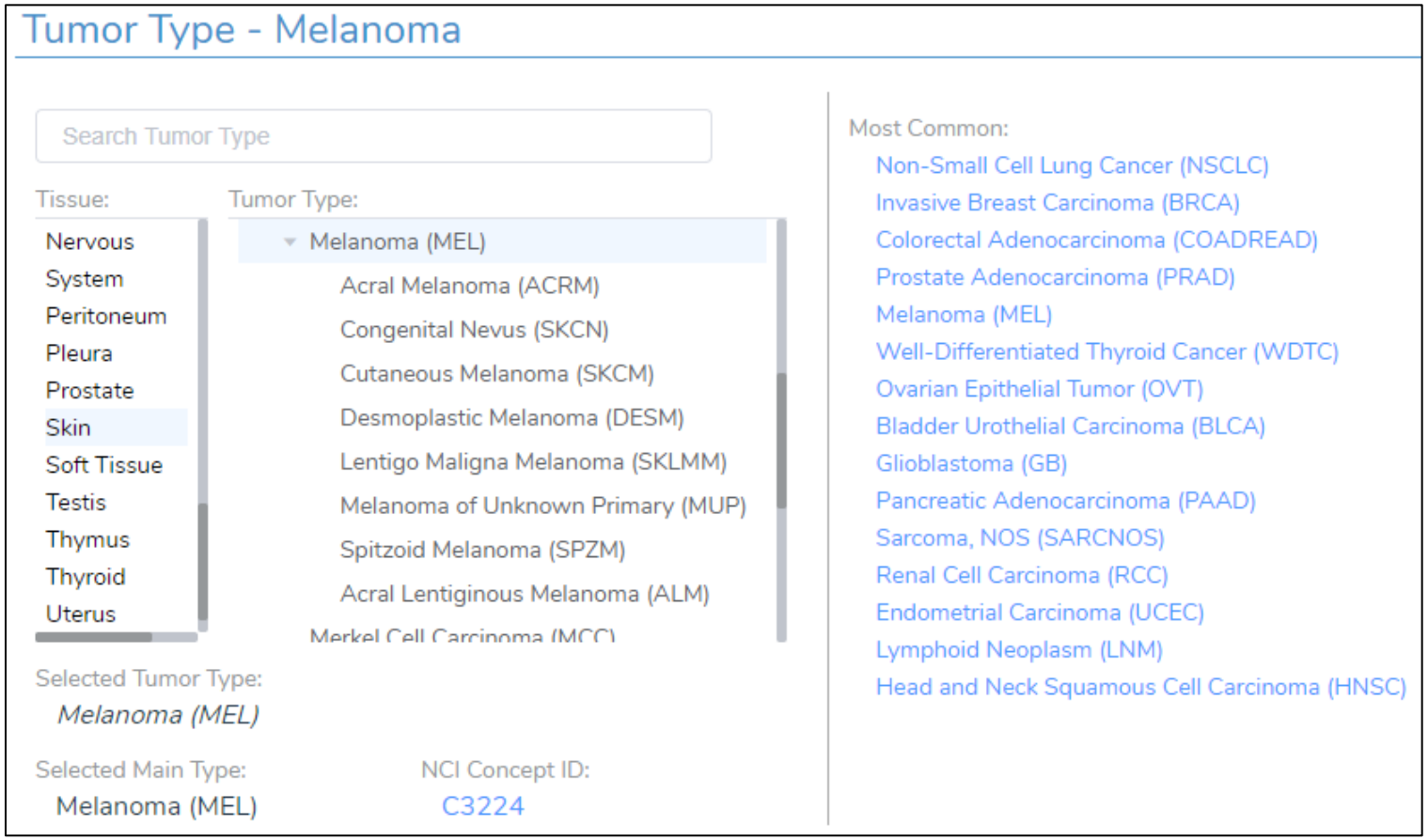
- 感受性と耐性それぞれに関連する薬剤のリストも得ることが可能



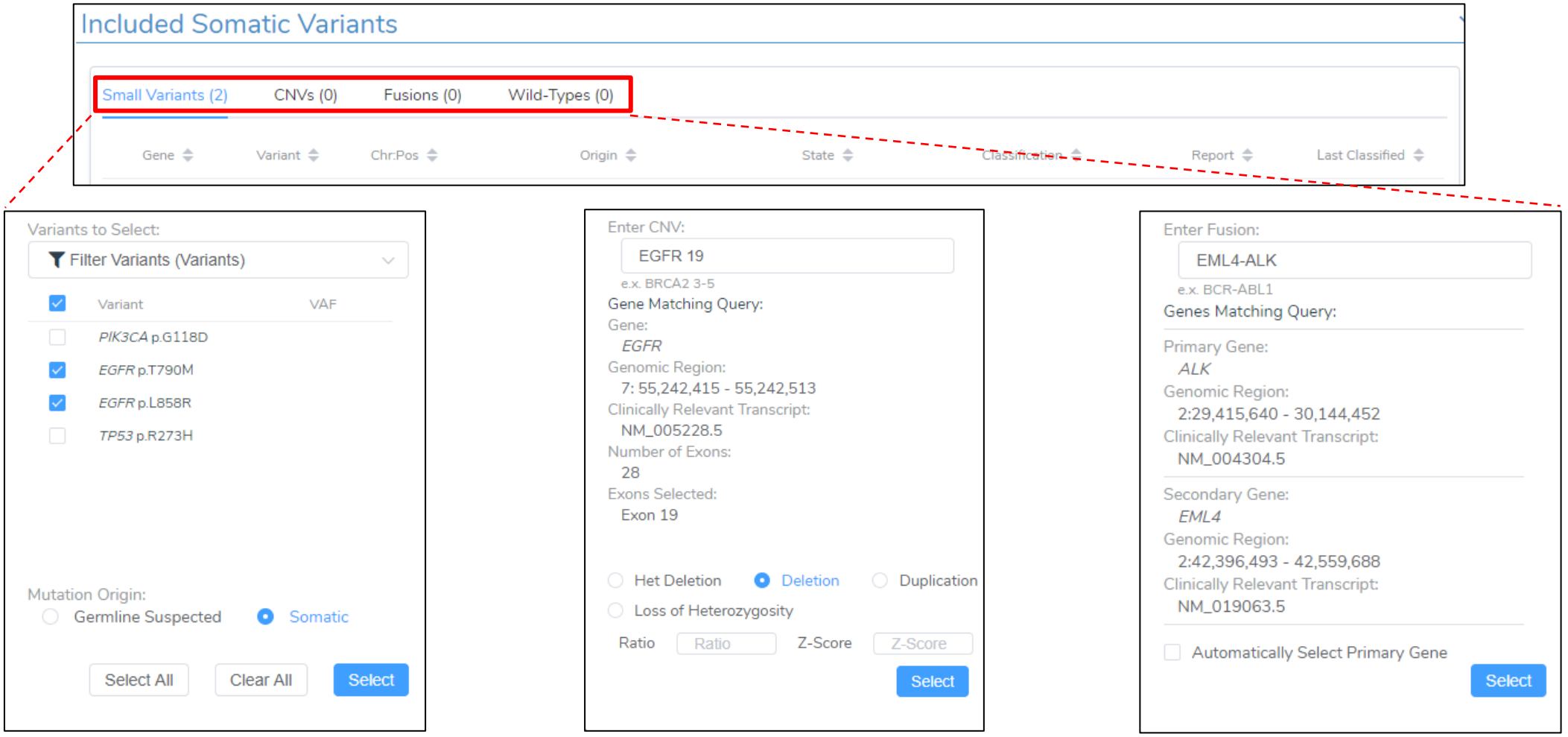


がん種の選択

- バイオマーカーの評価を行う前に、サンプルのがん種の選択が必要
- 代表的ながん種を選択するか、対象となる臓器名より、手動で検索することも可能



バイオマーカーの選択



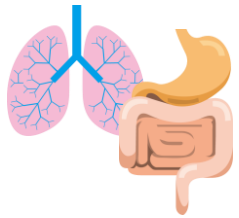
✓ Small Variant

✓ CNV

✓ Fusion Gene

- 評価に用いるバイオマーカーは、VCFファイル内のバリアントを選択するか、またはバイオマーカー名を手動で入力
- バリアントでは体細胞／生殖細胞の選択や、CNVは重複／欠失の設定も行う

臨床的評価の実行手順



がん種情報

バイオマーカー

Golden Helix CancerKB

Biomarker	Tumor Type	Clinical Significance
EGFR L858R	Non-Small Cell Lung Cancer	Tier I Level A
TP53 R273H	Non-Small Cell Lung Cancer	Tier II Level C
RET Activating Mutation	Non-Small Cell Lung Cancer	Tier I Level A

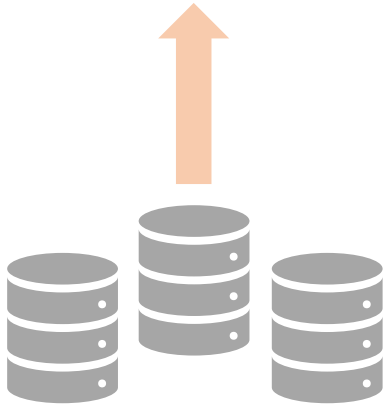
✓ エビデンスレベル分類

+

✓ 臨床的解釈

✓ 候補治療薬

✓ 論文リスト

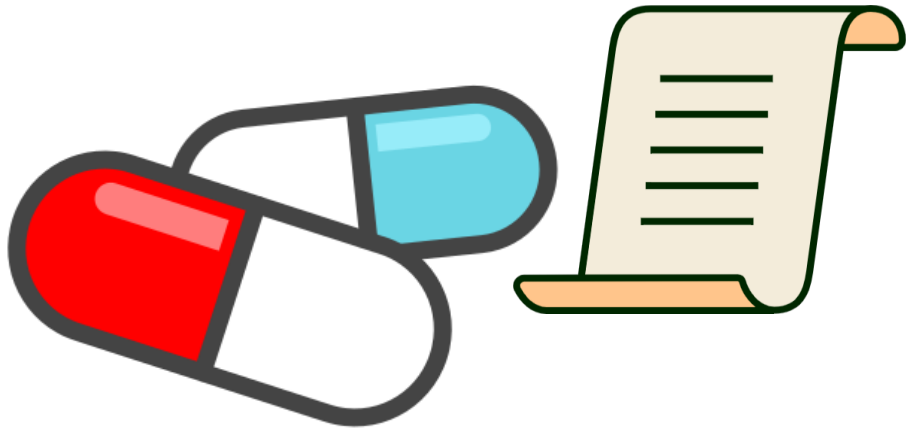


必要に応じて、他の知識ベースのデータを利用し、情報を編集

- がん種とバイオマーカーを入力すると、Golden Helix CancerKBに照合が行われ、自動的にエビデンスレベルや各種の臨床評価情報などが出力される
- Golden Helix CancerKBからの出力結果は、他の知識ベースの情報を参照しながら、編集が可能

薬剤と臨床試験情報

Drug & Clinical Trials Review



■ 候補治療薬の詳細と、臨床試験情報の確認を行う

- 薬剤情報
  （DrugBank）
  - ✓ Drug Description
  - ✓ Indications and Use
  - ✓ Pharmacodynamics
  - ✓ Mechanism of Action
- 臨床試験情報
  （NCI Cancer Clinical Trials）
  - ✓ Phase/status
  - ✓ Study type and location
  - ✓ Contact information
  - ✓ Start and finish date
  - ✓ Min-max age/gender

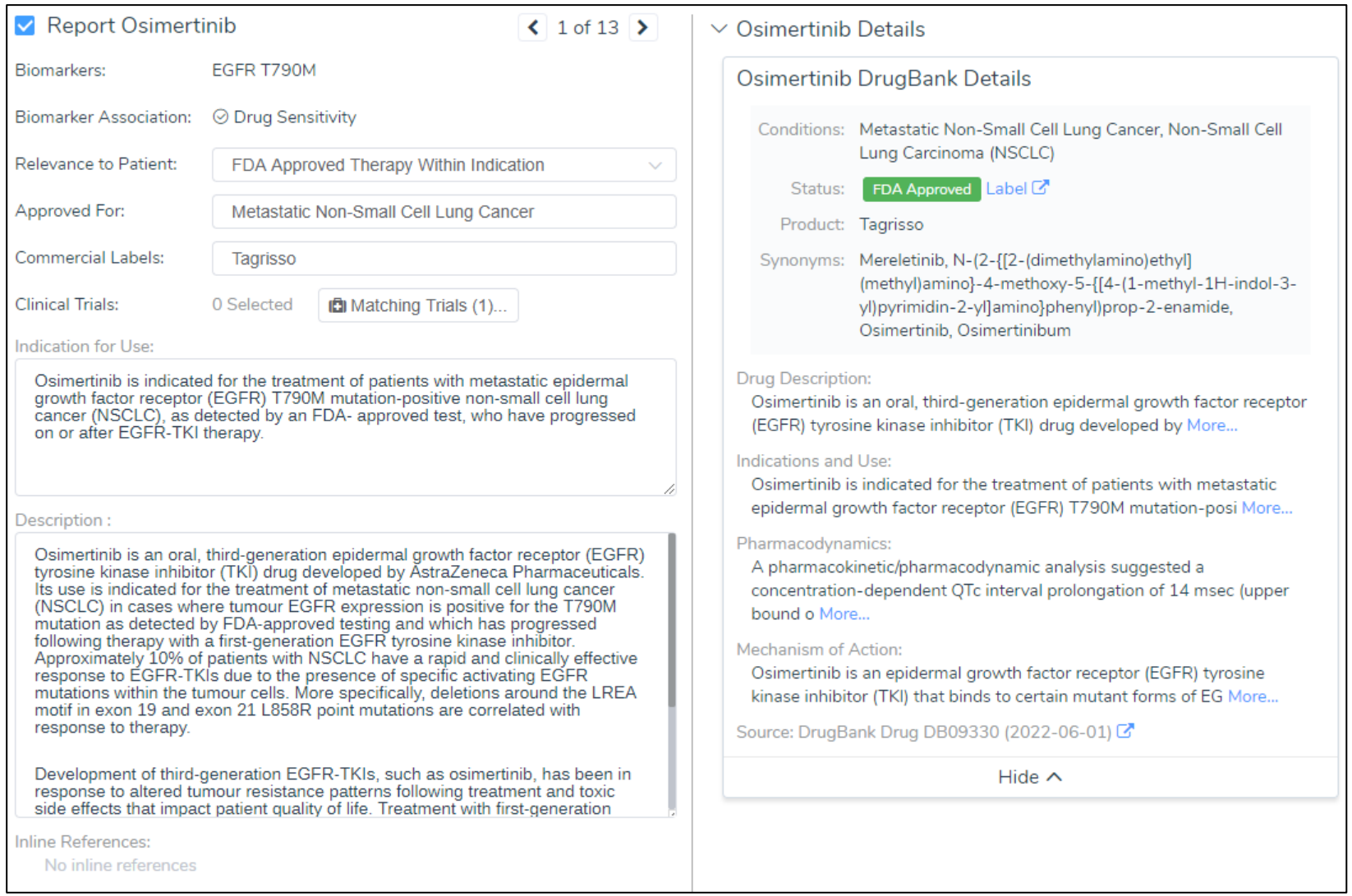
■ 臨床試験情報は、国名を指定しての検索が可能

治療オプションリスト

- 候補治療薬ごとに、FDA承認や臨床試験情報の一覧が表示される
- 国名を選択しておけば、選択国内で進行中の臨床試験の件数が表示される

薬剤の詳細情報

- 候補治療薬ごとに、DrugBankに登録されている薬剤の詳細情報をダッシュボード画面で確認が可能
- バイオマーカーの候補治療薬リストとともに、レポートに掲載することも可能

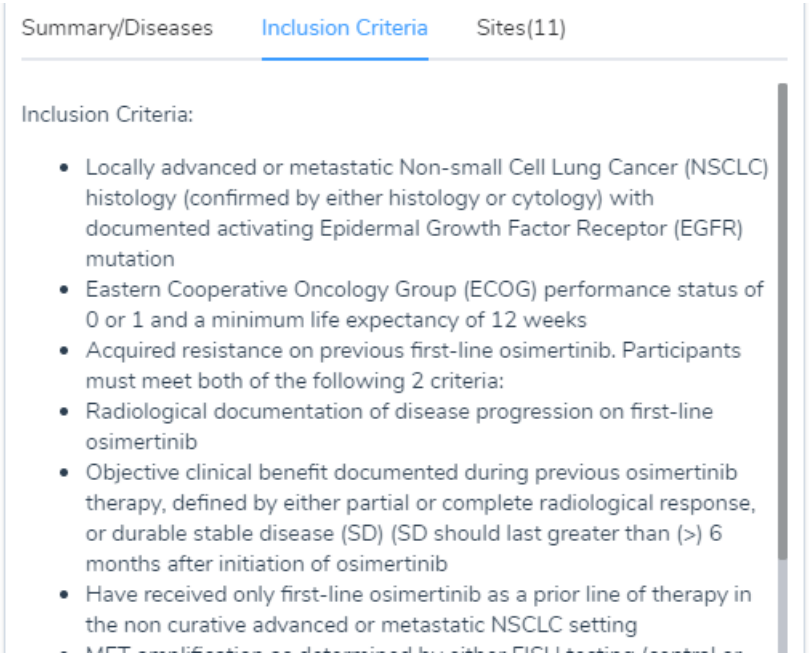


臨床試験の詳細情報

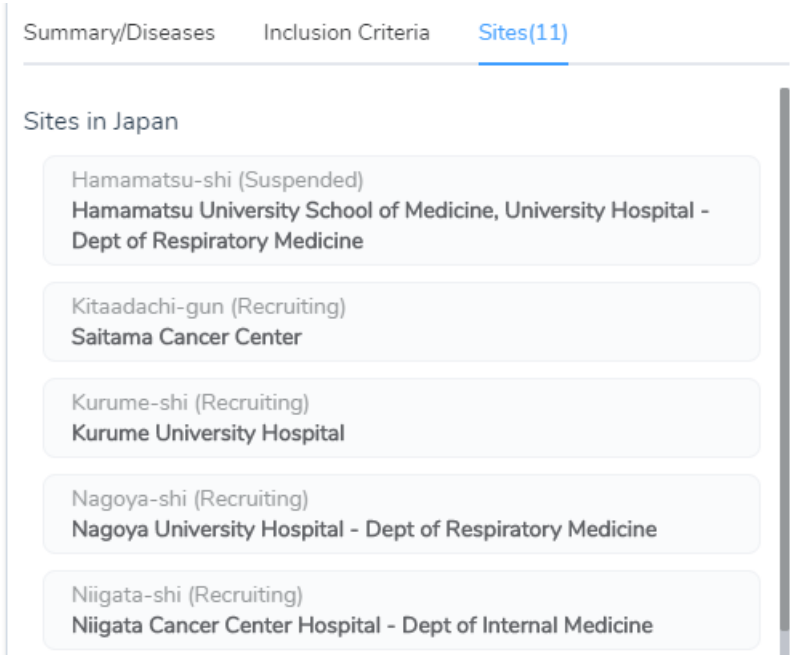
✓ 試験IDとタイトル

NCT03940703 A Study of Tepotinib Plus Osimertinib in Osimertinib Relapsed MET Amplified NSCLC (INSIGHT 2)
Nearest Location: Hamamatsu-shi (Hamamatsu University School of Medicine, Universit...)

✓ サマリー



✓ 対象者の基準

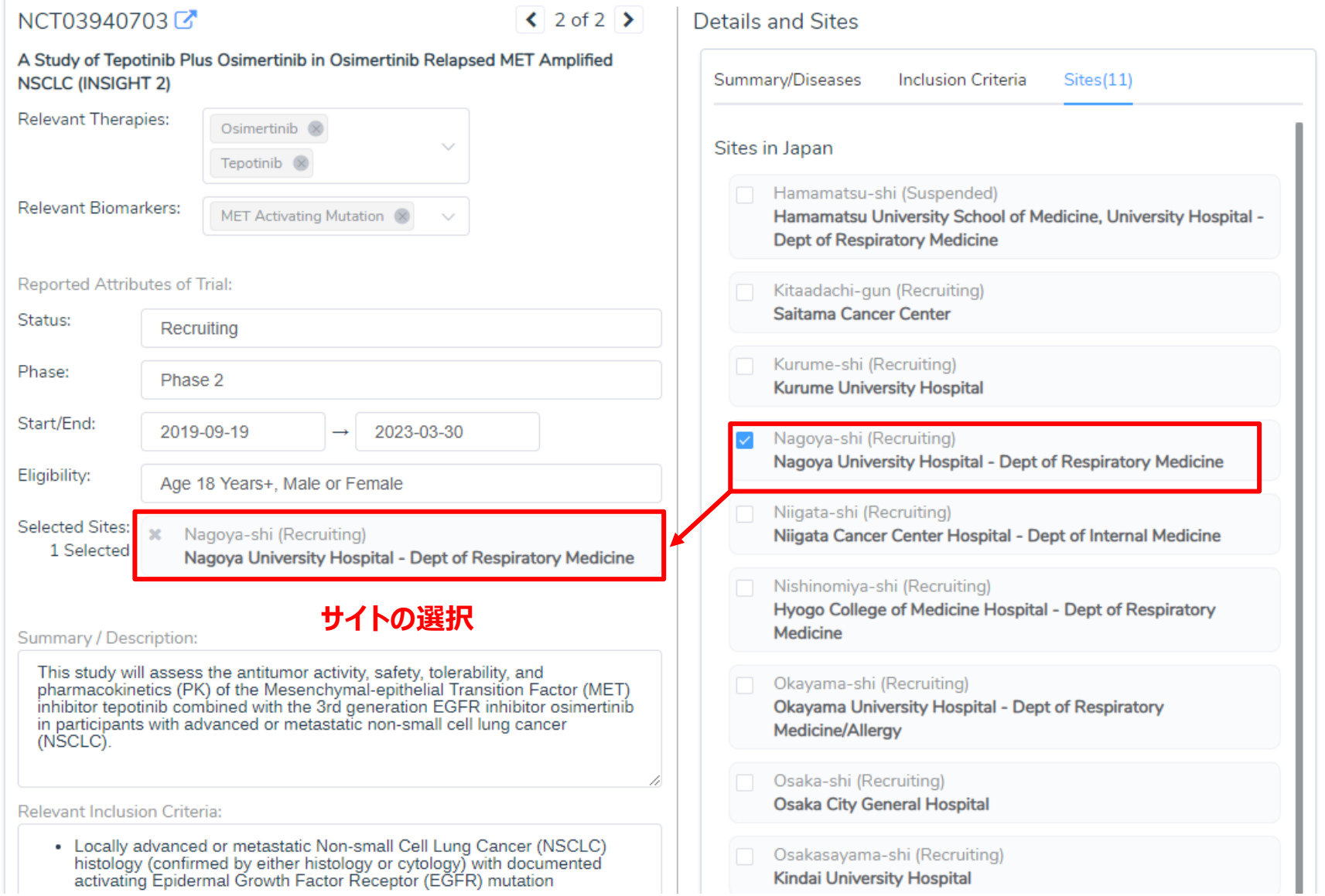


✓ 実施サイト

- 臨床試験については、試験IDやタイトルに加え、各種詳細情報も確認可能
- 試験サイトについては、リクルート中かどうかも表示される

実施サイトのレポート登録

- 臨床試験のレポート編集画面では、レポートに記載する実施サイトや対象基準の選択が可能



レポートコンテンツ

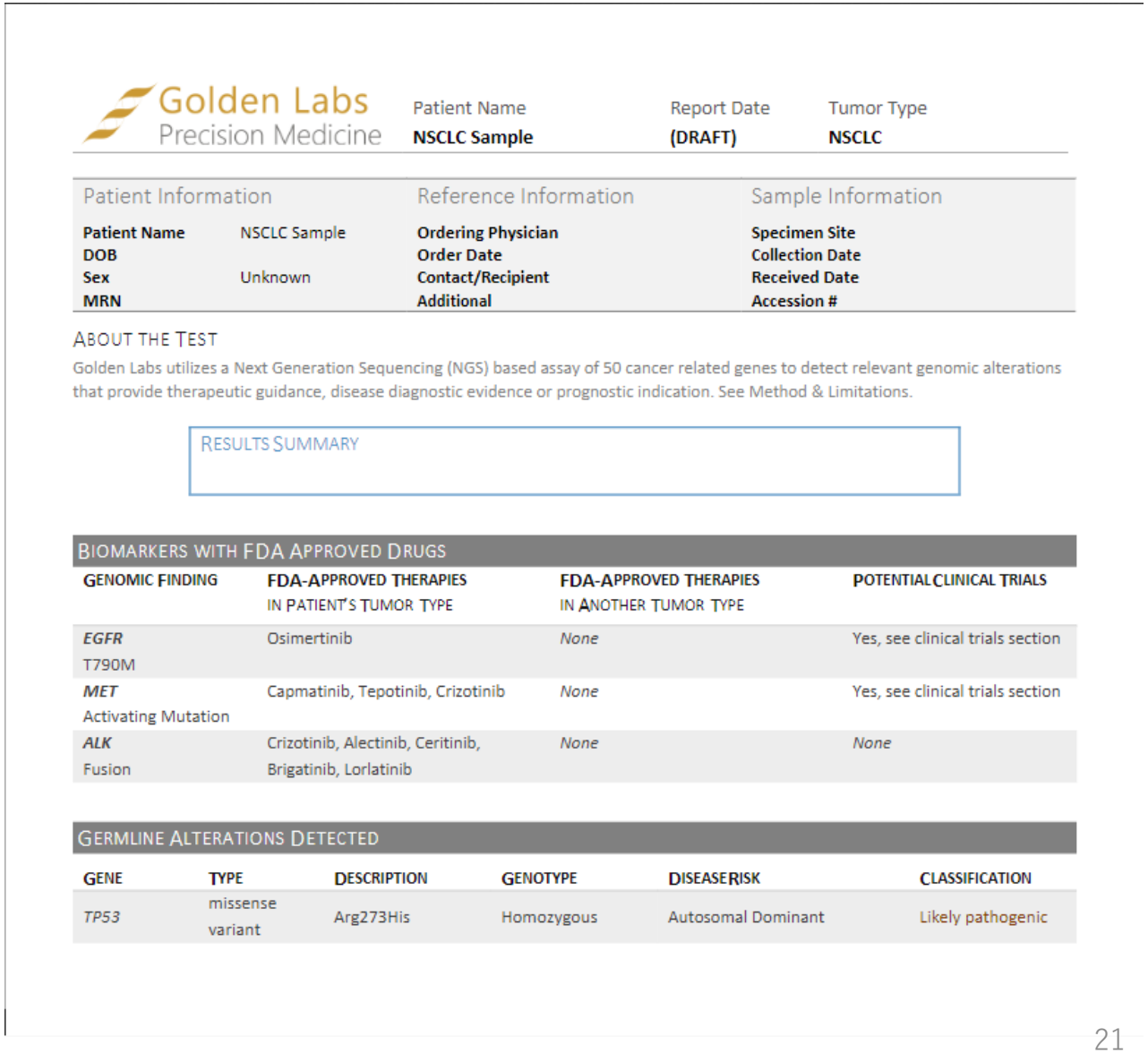
Report Generation

■ レポートを作成すると、以下のコンテンツが自動で収録され、項目の選択も可能

- Patient information
- Biomarkers
- Secondary germline
- Drugs and trials
- Coverage and sequence summary
- Citations

■ バイオマーカーについては、最大で以下の7セクションごとの臨床的解釈を、レポートに記載することが可能

- Gene Summary
- Outcomes & Frequency
- Biomarker Summary
- Clinical Evidence
  - ・Drug Sensitivity
  - ・Drug Resistance
  - ・Prognostic
  - ・Diagnostic



Golden Labs Precision Medicine

Patient Name	Report Date	Tumor Type
NSCLC Sample	(DRAFT)	NSCLC

Patient Information		Reference Information	Sample Information
Patient Name	NSCLC Sample	Ordering Physician	Specimen Site
DOB		Order Date	Collection Date
Sex	Unknown	Contact/Recipient	Received Date
MRN		Additional	Accession #

ABOUT THE TEST

Golden Labs utilizes a Next Generation Sequencing (NGS) based assay of 50 cancer related genes to detect relevant genomic alterations that provide therapeutic guidance, disease diagnostic evidence or prognostic indication. See Method & Limitations.

RESULTS SUMMARY

BIOMARKERS WITH FDA APPROVED DRUGS

GENOMIC FINDING	FDA-APPROVED THERAPIES IN PATIENT'S TUMOR TYPE	FDA-APPROVED THERAPIES IN ANOTHER TUMOR TYPE	POTENTIAL CLINICAL TRIALS
EGFR T790M	Osimertinib	*None*	Yes, see clinical trials section
MET Activating Mutation	Capmatinib, Tepotinib, Crizotinib	*None*	Yes, see clinical trials section
ALK Fusion	Crizotinib, Alectinib, Ceritinib, Brigatinib, Lorlatinib	*None*	*None*

GERMLINE ALTERATIONS DETECTED

GENE	TYPE	DESCRIPTION	GENOTYPE	DISEASE RISK	CLASSIFICATION
TP53	missense variant	Arg273His	Homozygous	Autosomal Dominant	Likely pathogenic

レポート記載例

EGFR

T790M

Tier I - Level A

Drug Sensitivity

Drug Resistance

Sensitivity to Drugs:
Osimertinib

Resistance to Drugs:
Erlotinib
Gefitinib
Afatinib
Dacomitinib

EGFR T790M Biomarker Summary:
EGFR p.T790M is an exon 20 missense mutation that is associated with acquired resistance to EGFR tyrosine kinase inhibitor (TKI) therapy in non-small cell lung cancer (NSCLC). *EGFR* p.T790M has been reported as an acquired mutation in approximately 60% of NSCLC patients with disease progression after initial response to first- or second-generation EGFR TKIs (PMID: 25979928, 23470965, 25271963, 24101047, 15737014, 17020982, 19589612). Within the EGFR ATP binding pocket, codon Thr790 represents a "gatekeeper" residue that regulates TKI binding. The precise mechanisms of *EGFR* p.T790M resistance remain unclear, although preclinical studies suggest that this mutation confers TKI resistance by increasing EGFR affinity for ATP and/or sterically hindering TKI binding to EGFR (PMID: 18227510, 15728811, 15897464, 17020982). The *EGFR* p.T790M mutation is generally detected together with *EGFR* sensitizing mutations such as exon 19 deletions (Ex19del) or p.L858R (PMID: 31562956, 30108370). In very rare cases (~0.5% of never-smokers with NSCLC), *EGFR* p.T790M has been identified in treatment-naïve NSCLC patients; in this context, *EGFR* p.T790M may be a germline mutation that is associated with predisposition to familial lung cancer (PMID: 16258541, 22588155, 24736066).

✓ バイオマーカー情報

Osimertinib
FDA Approved Therapy
Within Indication

FDA Approved

Drug Sensitivity

Approved For:
Metastatic Non-Small Cell Lung Cancer

Relevant Biomarker:
EGFR T790M

Commercial Labels:
Tagrisso

Indication for Use: Osimertinib is indicated for the treatment of patients with metastatic epidermal growth factor receptor (EGFR) T790M mutation-positive non-small cell lung cancer (NSCLC), as detected by an FDA- approved test, who have progressed on or after EGFR-TKI therapy.

Osimertinib Description:
Osimertinib is an oral, third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) drug developed by AstraZeneca Pharmaceuticals. Its use is indicated for the treatment of metastatic non-small cell lung cancer (NSCLC) in cases where tumour EGFR expression is positive for the T790M mutation as detected by FDA-approved testing and which has progressed following therapy with a first-generation EGFR tyrosine kinase inhibitor. Approximately 10% of patients with NSCLC have a rapid and clinically effective response to EGFR-TKIs due to the presence of specific activating EGFR mutations within the tumour cells. More specifically, deletions around the LREA motif in exon 19 and exon 21 L858R point mutations are correlated with response to therapy.
Development of third-generation EGFR-TKIs, such as osimertinib, has been in response to altered tumour resistance patterns following treatment and toxic side effects that impact patient quality of life. Treatment with first-generation EGFR-TKIs (gefitinib and erlotinib) has been associated with the development of resistance through activating mutations in the EGFR gene. Second-generation EGFR-TKIs (afatinib and dacomitinib) were then developed to be more potent inhibitors, although their use is associated with increased toxicity through nonspecific targeting of wild-type EGFR. In contrast, third-generation inhibitors are specific for the gate-keeper T790M mutations which increases ATP binding activity to EGFR and result in poor prognosis for late-stage disease. Furthermore, osimertinib has been shown to spare wild-type EGFR during therapy, thereby reducing non-specific binding and limiting toxicity.

✓ 薬剤情報

TP53
Homozygous p.R273H
NM_000546.6:c.818G>A

Likely pathogenic

Interpretation Disorder: Unspecified / All Highly Penetrant Disorders

ACMG Classification: Likely pathogenic

Interpretation: The missense variant NM_000546.6(TP53):c.818G>A (p.Arg273His) causes the same amino acid change as a previously established pathogenic variant. The variant is observed in one or more well-documented healthy adults. There is a small physicochemical difference between arginine and histidine, which is not likely to impact secondary protein structure as these residues share similar properties. 29 variants within 6 amino acid positions of the variant Arg273His have been shown to be pathogenic, while none have been shown to be benign. The Arg273His missense variant is predicted to be damaging by both SIFT and PolyPhen2. The arginine residue at codon 273 of TP53 is conserved in all mammalian species. The nucleotide c.818 in TP53 is predicted conserved by GERP++ and PhyloP across 100 vertebrates. For these reasons, this variant has been classified as Likely Pathogenic.

Technical Data for TP53 Arg273His:

Transcript and Coding Change	NM_000546.6:c.818G>A (p.Arg273His)
Location	17:7577120
Human Genome (GRCh37)	NC_000017.10: g.7577120C>T
Human Genome (GRCh38)	NC_000017.11: g.7673802C>T
dbSNP Identifier	rs28934576
ClinVar Variant ID	12366
Ashkenazi Jewish Allele Frequency	1/10072 (0.01%)
NGS Reads Supporting Change	100.00%

✓ 生殖細胞系列バリアント情報

NCT03940703
Started: 2019-09-19
Ends: 2023-03-30

Phase 2

Relevant Therapy:
Osimertinib
Tepotinib

Relevant Biomarker:
MET Activating Mutation

Nearby Sites:

Nagoya-shi
Nagoya University Hospital - Dept of Respiratory Medicine

A Study of Tepotinib Plus Osimertinib in Osimertinib Relapsed MET Amplified NSCLC (INSIGHT 2)

This study will assess the antitumor activity, safety, tolerability, and pharmacokinetics (PK) of the Mesenchymal-epithelial Transition Factor (MET) inhibitor tepotinib combined with the 3rd generation EGFR inhibitor osimertinib in participants with advanced or metastatic non-small cell lung cancer (NSCLC).

Eligibility: Age 18 Years+, Male or Female

✓ 臨床試験情報

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